(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 217 102**
A1

(12) # EUROPEAN PATENT APPLICATION

(21) Application number: 86111585.5

(22) Date of filing: 21.08.86

(51) Int.Cl.⁴: C 12 N 15/00
C 07 H 21/04, C 07 H 21/02
C 12 N 1/20, C 12 N 9/00
C 12 Q 1/00, G 01 N 33/53
C 07 K 7/10, A 61 K 39/395
//(C12N1/20,C12R1:19)

(30) Priority: 28.08.85 IL 76233
08.04.86 IL 78445

(43) Date of publication of application:
08.04.87 Bulletin 87/15

(84) Designated Contracting States:
BE CH DE FR GB IT LI NL SE

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(54) **Interferon-induced (2'-5') oligo a synthetase gene, mRNA, cDNA and enzymes having (2'-5') oligo a synthetase activity.**

(57) The present invention relates to a human DNA sequence coding for an enzyme having (2'–5') oligo A synthetase activity, a 1.6 kb RNA and a 1.8 kb RNA being complementary to the mentioned DNA sequences, to a DNA transfer vector, comprising an inserted DNA sequence consisting essentially of the mentioned DNA sequence, furthermore to a micro-organism which has been transformed by the said transfer vector and is capable of expressing an enzyme having the (2'–5') oligo A synthetase activity. The present invention furthermore provides a method of monitoring the response of a patient to an interferon, which comprises measuring the concentration of (2'–5') oligo A synthetase mRNA in cells or body fluids of the patient by hybridizing the mRNA to DNA complementary thereto. In addition there are provided antigenic peptides and antibodies raised against the antigenic peptides which recognises and immunoprecipitates (2'–5') oligo A synthetase.

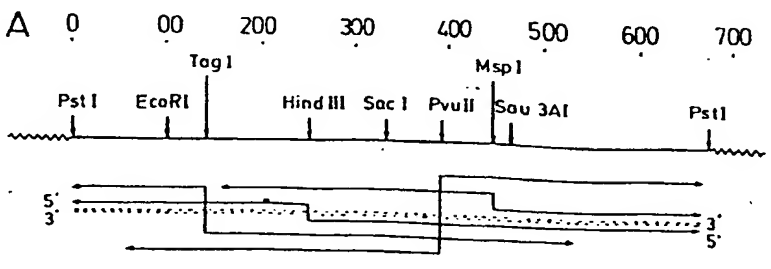

FIG. 1A

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                                        30                                  50
TTT CGG ACG GTC TTG GAA TTA GTC ATA AAC TAC CAG CAA CTC TGC ATC TAC TGG ACA AAG
PHE ARG THR VAL LEU GLU LEU VAL ILE ASN TYR GLN GLN LEU CYS ILE TYR TRP THR LYS
                                        90                                 120
TAT TAT GAC TTT AAA AAC CCC ATT ATT GAA AAG TAC CTG AGA AGG CAG CTC ACG AAA CCC
TYR TYR ASP PHE LYS ASN PRO ILE ILE GLU LYS TYR LEU ARG ARG GLN LEU THR LYS PRO
                                       150                                 190
AGG CCT GTG ATC CTG GAC CCG GCG GAC CCT ACA GGA AAC TTG GGT GGT GGA GAC CCA AAG
ARG PRO VAL ILE LEU ASP PRO ALA ASP PRO THR GLY ASN LEU GLY GLY GLY ASP PRO LYS
                                       210                                 230
GGT TGG AGG CAG CTG GCA CAA GAG GCT GAG GCC TGG CTG AAT TAC CCA TGC TTT AAG AAT
GLY TRP ARG GLN LEU ALA GLN GLU ALA GLU ALA TRP LEU ASN TYR PRO CYS PHE LYS ASN
                                       270                                 300
TGG GAT GGG TCC CCA GTG AGC TCC TGG ATT CTG CTG GTG AGA CCT CCT GCT TCC TCC CTG
TRP ASP GLY SER PRO VAL SER SER TRP ILE LEU LEU VAL ARG PRO PRO ALA SER SER LEU
                                       330                                 360
CCA TTC ATC CCT GCC CCT CTC CAT GAA GCT TGA GAC ATA TAG CTG GAG ACC ATT CTT TCC
PRO PHE ILE PRO ALA PRO LEU HIS GLU ALA
                                       390                                 820
AAA GAA CTT ACC TCT TGC CAA AGG CCA TTT ATA TTC ATA TAG TGA CAG GCT GTG CTC CAT
                                       450                                 830
ATT TTA CAG TCA TTT TGG TCA CAA TCG AGG GTT TCT GGA ATT TTC ACA TCC CTT GTC CAG
                                       510
AAT TCA TTC CCC TAA GAG TAA TAA TAA ATA ATC TCT AAC ACC AAA
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FIG.1B

Many of the biological effects of interferon appear to be mediated by the induction of new mRNAs and proteins in cells exposed to IFNs (for review: Revel, 1984; Lebleu and Content, 1982; Baglioni and Nilsen, 1983). Among these IFN-iduced proteins two groups appear particularly important: 1) translation regulatory enzymes (ds RNA dependent protein kinase and (2'-5') oligo A synthetase, (2'-5') oligo A-activated nuclease, 2'-phosphodiesterase) and 2) cell surface antigens (HLA-A, B, C, B2-microglobulin, HLA-DR). Other cellular and excreted proteins play probably important roles as well (Weil et al, 1983; Chebath et al, 1983; Wallach et al, 1983). With the exception of the HLA genes (Malissen et al, 1982; Schamboeck et al, 1983), the structure and sometimes the function of the IFN-induced proteins is unknown and so is the mechanism by which IFNs activate specifically these genes. To address these questions, several cDNAs from IFN-iduced genes have been recently cloned (Chebath et al, 1983; Merlin et al, 1983; Friedman et al, 1984; Samanta et al, 1984). In particular, the cDNA and gene coding for the human (2'-5') oligo A synthetase was studied, a ds RNA-activated enzyme that converts ATP into ppp(A2'pA)n oligomers (Kerr and Brown, 1978) which in turn bind to and activate the latent RNase F (Schmidt et al, 1978). The (2'-5') oligo A synthetase is strongly induced in cells by all three types of human IFNs, and its increase is a good marker of IFN activity (Wallach et al, 1982). The enzyme is induced during differentiation of hematopoietic cells, and denotes an autocrine secretion of IFN-β (Yarden et al, 1984). The enzyme is similarly induced late in the S phase of synchronized embryo fibroblasts (Wells and Mallucci, 1985). The enzyme activity drops when cell growth starts (Etienne-Smekens et al, 1983; Creasey et al, 1983) and appears to be invol-

ved in the antigrowth effect of IFN (Kimchi et al, (1981). Deficiency in the (2'-5') oligo A synthetase or in the (2'-5') oligo A-activated RNase F have also been correlated with partial loss of the antiviral effects of IFNs (Salzberg et al, 1983; Epstein et al, 1981), although this is probably not the only mechanism by which IFN inhibits virus growth (Lebleu and Content, 1982). The (2'-5') oligo A nucleotides have been detected in many eukaryotic cells and even in bacteria (Laurence et al, 1984) and the synthetase is likely to be a wide-spread enzyme. The enzyme has been purified from mouse (Dougherty et al, 1980) and human cells (Yand et al, 1981; Revel et al, 1981); a large and a small form of the enzyme have been observed (Revel et al, 1982; St. Laurent et al, 1983) but their structures were not elucidated.

The (2'-5') oligo A synthetase, induced in cells exposed to IFNs (Hobanessian et al, 1977; Zilberstein et al, 1978) has a number of unusual properties. Its main activity is the synthesis from ATP of 5'triphosphorylated short oligo A chains (of up tc 15 A, with mainly dimers to pentamers), but in contrast to other RNA polymerases, it adds adenylate or one other nucleoide specifically to the 2'OH of adenylate in oligo A (Kerr and Brown, 1978; Samanta et al, 1980), or to other (oligo)nucleotides with a free 2'OH adenylate such as NAD (Ball, 1980) or even tRNA (Ferbus et al, 1981). To be active, the enzyme has to bind to double-stranded RNA stretches of minimum 50 bp (Minks et al, 1979), and must therefore possess several binding sites: for nucleoside triphosphates, for 2'OH adenosine polynucleotides and for double stranded RNA. The enzyme binds to 2', 5' ADP-Sepharose (Johnston et al, 1980), to poly (rI)(rC)-agarose (Hovanessian et al, 1977) and to Cibacron Blue-Sepharose (Revel et al, 1981). In different cells, the (2'-5') oligo A synthetase activity is in the cytosol (Revel et al, 1981) or in ribosomal salt washes (Dougherty et al, 1980), as well as in the nuclear sap (Nilsen et al, 1982b) and even in large amounts in the nuclear matrix. It is notable that cellular RNAs can replace poly (rI)(rC) for activation of the enzyme (Revel et al, 1980) and the synthetase may even have a role in Hn RNA processing (Nilsen et al, 1982a). Some (2'-5') oligo A synthetase is bound to plasma membranes and can be incorporated in budding virions (Wallach and

Revel, 1980). These complex interactions may ensure a localized action of the (2'-5') oligo A system (Nilsen and Baglioni, 1983) and explain its multiple suggested roles in normal and virus-infected cells. The synthetase amounts to less than 0.1% of the proteins in IFN-treated cells, and its structure could not be determined directly.

It is possible to use measurements of (2'-5') oligo A synthetase levels to determine whether cells in vitro or in vivo have been exposed to IFN and respond to it. This measurement can be used as an assay for IFN in unknown solutions, by exposing cells to said solutions and determining the increase in (2'-5') oligo A synthetase levels (Revel et al., US patent 4,302,533). The measurement can also be used to establish whether IFN is produced in increased amounts in whole organisms including man.

CLINICAL APPLICATIONS OF (2'-5') OLIGO A SYNTHETASE MEASUREMENTS

It has been established that the (2'-5') oligo A synthetase level is rather constant in peripheral blood mononuclear cells (PBMC) of healthy individuals (Schattner et al, 1981b). An increase in (2'-5') oligo A synthetase is seen in PBMC of patients with acute viral infections (Schattner et al., 1981b; Schoenfeld et al., 1985), with persistent viral infections (Wallach et al., 1982), with autoimmune diseases and with a number of other syndromes suspected of infectious origin such as Jacob-Kreuzfeld disease (Revel et al., 1982). The basal (2'-5') oligo A synthetase level is lower in granulocytes but large increase in viral infections are seen (Schattner et al., 1984). Increase of (2'-5') oligo A synthetase enzyme in PBMC of AIDS patients was recently reported (Read et al., 1985). In animal models, it was shown that increase in (2'-5') oligo A synthetase level is rapid and more constant than appearance of IFN in the blood (Schattner et al. 1982a). (2'-5') oligo A synthetase remains high for several weeks while the IFN peak is transient.

The (2'-5') oligo A synthetase increases during differentiation of haematopoietic cells as a result of autocrine secretion of IFN-β

(Yarden et al., 1984). Decreased (2'-5') oligo A synthetase levels are seen in acute leukemias with numerous blast cells (Wallach et al. 1982; Schattner et al, 1982b).

Another important application of (2'-5') oligo A synthetase measurements is in the monitoring of patients under IFN therapy. Besides clinical changes, it is possible to establish that the patients respond to IFN by measuring the PBMC (2'-5') oligo A synthetase level which increases 5-10 fold during systemic IFN-α as well as β treatment (Schattner et al., 1981a , Schoenfeld et al., 1984). It is clear that assay of other IFN-induced activities or molecules can be used as well as the assay of the (2'-5') oligo A synthetase enzyme, but this method has been the most widely used (Read et al., 1985; Merritt et al., 1985). In all these studies, the enzymatic assay measuring the conversion of ATP into (2'-5') (A)n oligomers has usually been employed. (Revel et al., US patent 4,302,533).

To detect the (2'-5') oligo A synthetase anti-(2'-5') oligo A synthetase peptide antibodies can be used. For the obtaining of antibodies to (2'-5') oligo A synthetase, peptide sequences can be chosen from the total amino acid sequences of different (2'-5') oligo A synthetases, to serve as antigens for the induction of antibodies against the native (2'-5') oligo A synthetase molecule. Rabbits were injected subcutaneously with the antigenic peptides in order to produce the antibodies. Subsequent boostings were done and continued until maximal antibody response.

The present invention concerns human DNA encoding an enzyme having (2'-5') oligo A synthetase activity. One form of the DNA has the nucleotide sequence set forth in Figure 7A. Another form of the DNA has the sequence of nucleotides 1-1322 set forth in Figure 7A which overlaps with the sequence of nucleotides 901-1590 set forth in Figure 7B.

An enzyme having (2'-5') oligo A synthetase activity has the amino acid sequence set forth in Figure 7A. Another enzyme having (2'-5')

oligo A synthetase activity has the sequence of amino acids 1-364 set forth in Figure 7A which overlaps with the sequence of amino acids 290-400 set forth in Figure 7B.

A 1.6 kb and 1.8 kb RNA having nucleotide sequences complementary to the nucleotide sequences in Figures 7A and 7B have been isolated.

A method of monitoring the response of a patient to an interferon comprises measuring the concentration of (2'-5') oligo A synthetase mRNA in cells or body fluids of the patient by hybridizing to the mRNA DNA complementary thereto.

Antigenic peptides of the present invention have an amino acid sequence contained within the amino acid sequences set forth in Figures 7A and 7B. Antibodies raised against these antigenic peptides recognize and immunoprecipitate (2'-5') oligo A synthetase.

A method of monitoring interferon activity in a subject comprises measuring the amount of (2'-5') oligo A synthetase in a cell or body fluid of the subject at predetermined time intervals, determining the differences in the amount of said synthetase in the cell or body fluid of the subject within the different time intervals, and determining therefrom the amount of synthetase in the cell or body fluid of the subject and thereby the interferon activity of the subject. The synthetase may be measured by contacting the synthetase with an antibody of the present invention so as to form a complex therewith and determining the amount of complex so formed.

Furthermore, the present invention concerns two antibodies for (2'-5') oligo A synthetase which can be obtained by preparing antigens which comprise a partial amino acid sequence of (2'-5') oligo A synthetase. The anti-(2'-5') oligo A synthetase peptide antibodies can be used to detect the enzyme.

Brief description of the Figures

Figure 1 depicts the structure and sequence of (2'-5') oligo A synthetase E_1 cDNA clone 174-3:

Figure 1A depicts the restriction map of E_1 cDNA clone 174-3. The insert base pairs are numbered in the same direction as pBR322 DNA. The pBR Eco R1 site is on the right. Both strands of the insert (dotted lines) were sequenced (Maxam & Gilbert, 1980) from the restriction sites indicted by the vertical lines. The coding strand is 5' to 3' from right to left. Following the right Pst1 site there were 17G and 72T, followed by the dinucleotide GA and the 3T of the sequence shown in (B) which are therefore not part of the tails. At the 3' end, tails of 45A and 10C preceded the left Pst 1 site.

Figure 1B depicts the nucleotide sequence having the longest coding frame. The first T is nucleotide 92 following the tails of the insert (right end in A). The Sau $3A_1$ site and the Eco R1 of the insert are at positions 129 and 480 respectively of the sequence shown.

Figure 2 depicts the size and induction of E_1 specific mRNAs in SV80 and Namalva cells:

Figure 2A depicts the hybridization of nick translated $[^{32}P]$-cDNA of clone E_1 to electrophoretic blots of denatured poly A^+-RNA from SV80 cells. The RNAs were prepared at the indicated hour after IFN-beta-1 addition. The apparent size of the RNA is indicated on the autoradiography. Left lane, rRNA markers.

Figure 2B is the same as 2A with RNA from Namalva cells treated with IFN-alpha for the indicated time. Left lane: rRNA markers.

Figure 3 depicts the characterization by hybridization to RNA blots of recombinant plasmid clone C56, harbouring cDNA for an IFN-induced mRNA Poly(A)$^+$ RNA from IFN-treated SV80 cells (I) or from non-treated cells (C), 7 micrograms were electrophoresed on agarose gels and after blotting to nitrocellulose were hybridized to nick-translated [^{32}P]-plasmid DNA of either the C56 clone, a human HLA cDNA clone or a rat tubulin cDNA clone. Exposure was for 48 h. Position of radioactive 18S of ribosomal RNA marker is indicated.

Figure 4 depicts the partial restriction map and nucleotide sequence of the C56 450 bp insert. The C56 plasmid was digested with Hind 3, end-labelled with alpha-[^{32}P]-dCTP by the DNA polymerase I-large fragment (Klenow enzyme, Boehringer) and the Hind 3 - Pst 1 fragments were separated on a 1% agarose gel. In order to sequence the complementary strand, the plasmid was 5'-labelled at the Bgl2 site with gamma [^{32}P]-ATP by the T_4-polynucleotide kinase (Biolabs) and the Bgl 2 - Pst 1 fragments were isolated. Sequencing was made by the Maxam and Gilbert technique. Sequence of coding strand A (right to left) is shown in the lower panel. The two first thymidylic residues of the sequence of strand A probably correspond to the AT tail as indicated in the upper diagram.

Figure 5 depicts the time course of the induction of C56 mRNA by IFN:

Figure 5A depicts Poly (A)$^+$RNA, 7 micrograms, from Namalva cells treated with IFN-alpha 1000 U/ml for the indicated times were electrophoresed on agarose gels and, after blotting, were hybridized with nick-translated [^{32}P]-C56 plasmid DNA.

Figure 5B depicts Poly (A)$^+$RNA, 7 micrograms, from SV80 cells treated with 200 U/ml IFN-beta for the indicated times. The asterisk indicates an RNA sample from cells treated with IFN-beta-1 purified on monoclonal antibody column (2×10^8 U/mg).

Figure 5C depicts Poly (A)$^+$RNA, 1 microgram, from SV80 cells treated as in (5B), was hybridized in liquid with 3' end-labelled fragment I of C56 DNA (see Fig. 4). The hybrids were treated

with S_1-nuclease and analyzed on denaturing gels. The mRNA-hybridized probe (⟶) is shorter than the self-reassociated probe (----→).

Figure 6 depicts the restriction map of cDNAs for the 1.6 and 1.8 kb (2'-5') oligo A synthetase mRNAs.

Figure 6A depicts the map of the 1.6 kb cDNA. The position of the E1 cDNA (Merlin et al., 1983) and of the lambda gt 10 cDNAs is shown. pA is the polyadenylation site. The exon limits are shown by vertical dotted lines. The size of the genomic DNA fragments carrying each exon are given in parentheses. The vertical arrow shows the position of the additional splice site in the 1.8 kb RNA. The strategy for sequencing the 9-21 and 5-21 cDNAs is indicated. The sequence from the 3' Eco R1 site (E) to the Pst 1 site (P) was determined in the E1 cDNA (Merlin et al., 1983).

Figure 6B depicts a map of the 1.8 kb cDNA. the lambda gt10 clone 48-1 was isolated using the Pst1-Pst1 genomic fragment containing exon 8 of the 1.8 kb RNA (Fig. 9). Exons are numbered as for the 1.6 kb E cDNA. The truncated exon 7 is designated 7a.

Figures 7A and B depict the nucleotide sequences of the two (2'-5') oligo A synthetase cDNAs. The nucleotides of the 1.8 kb cDNA clone 48-1 are numbered as for the 1.6 kb cDNA clone 9-21. Amino acid numbering is given in parantheses. Translation starts at the first or second codon of the ATGATG sequence. Limits between exons are shown by vertical bars. (Glycos.) indicates a possible glycosylation site in E18. Single base variations, possibly allelic differences, were detected between clones or genomic DNA in the 1.6 kb sequence at 376 (T for C), 525 (G for A), 807 (G for C), 811 (A for G); in the 1.8 kb sequence at 1087 (G for A), 1115 (G for C).

Figure 8 depicts the hydropathy plot of the C-termini of the E16 and E18 (2'-5') oligo A synthetases. The computer program of Kyte and

Doolittle (1982) was used. Hydrophobic regions are over the midline. The acidic region in E18 corresponds to amino acids 353 to 358 in Figure 7.

Figure 9 depicts the restriction map of the human (2'-5') oligo A synthetase gene. A map constructed from three overlapping genomic clones is shown with the position of the 7 exons of the 1.6 kb RNA and the additional 8th exon of the 1.8 kb RNA (black bars). The insert shows a Southern blot of genomic DNA with the 48-1 cDNA as probe. Slot 1, Daudi DNA; slot 2, diploid fibroblast FS11 DNA.

Figure 10 depicts the promoter region of the human (2'-5') oligo A synthetase gene. A restriction map of the Sph1-Sph1 0.85 kb fragment from the 4.2 kb EcoR1 genomic DNA segment in Figure 9 is shown. The 5' end of the mRNAs is marked as cap.

Figure 11 depicts the sequence of the human (2'-5') oligo A synthetase promoter region. The sequence of the Sau3a-Hpa1 segment shown in Figure 10, aligned for comparison with the promoter region of the human IFN-beta-1 gene (Degrave et al., 1981). Numbering is from the presumed cap site. A purine rich transcription regulatory sequence around -75 in the IFN-beta-1 promoter (Zinn et al., 1983), repeated at -10, is underlined. The TATA box is doubly underlined.

Figure 12 depicts the SDS-acrylamide gel electrophoresis of ^{35}S-methionine labelled proteins from IFN treated WISH cells immunoprecipitated by antiserum to synthetic peptides.

Figure 13 depicts the expression of E16 cDNA in E. coli. Extracts of E. coli lysogen Agt11-E16 induced by IPTG at 42°C were assayed on poly (rI) (rC) agarose beads for (2'-5') oligo A synthesis. Cont = extracts of E. coli with E16 cDNA in opposite orientation to lac Z gene. Nam = extracts of IFN-treated Namalva cells. Electrophoresis at pH 3.5 of alkaline phosphatased ^{32}P-a-ATP labelled products are shown.

Figure 14 depicts the rapid method for assay of (2'-5') oligo A synthetase RNAs in human peripheral white blood cells.

Figure 15 depicts the quick cell blot for (2'-5') oligo A synthetase E RNAs in human PBMC according to the method of Figure 14. Indicated number of cells and IFN (16 H treatment) were used. Autoradiography with ^{32}P-cDNA.

Figure 16 depicts the (2'-5') oligo A synthetase activity which is adsorbed on anti-B and anti-C IgG-Protein A-Sepharose was measured as described in Example . The scheme underneath shows the position of peptide B and peptide C in the two (2'-5') oligo A synthetase forms E16 and E18 sequenced by Benech et al. (1985b). The blackened area indicates the part of E18 which differs from the E16 molecule.

Figure 17 depicts electrophoresis and immunoblotting of extracts from human cells as described in Example 2. The position of the 4 forms of (2'-5') oligo A synthetase is indicted by the numbers on the right of each blot. M = ^{14}C-protein molecular weight markers. IFN treatment is indicated by +.

Figure 18 depicts electrophoretic immunoblots of extracts from human SV80 cells with anti-(2'-5') oligo A synthetase peptide B. Left: crude cytoplasmic extract (S1.5), cell sap (S100) and Microsomes (P100). Right: Na deoxycholate 10% extract of microsomes (DOC-soluble), high salt wash of microsomes (RWF) and microsomal pellet after salt extraction (Microsomes-KCl).

Figure 19 depicts the fractionation of S100 and high-salt wash of microsomes (RWF) on DEAE-cellulose and carboxymethyl-cellulose, followed by glycerol gradient. The (2'-5') oligo A synthetase profile and protein detected by anti-B are shown below the gradients. Electrophoretic immunoblot show fraction CM (CM-cellulose eluate from the S100 proteins non-adsorbed to DEAE-cellulose) on the left blot. On the right-hand blot fraction, DE (DEAE-cellulose eluate of RWF) and fraction GG (heavy peak of 80-100 kd from glycerol gradient of fraction DE).

Figure 20 depicts the double stranded RNA requirements of various forms of (2'-5') oligo A synthetase from SV80 cells. Fractions are labelled as in Figure 19. Enzymatic activity measured at indicated concentrations of poly (rI)(rC).

Figure 21 depicts the radio-immunoassay of (2'-5') oligo A synthetase with anti-B IgG and ^{125}I-Protein A as described in Example 3. Auto-radiography is shown. Cells treated for 16 hours with 500 U/ml IFN-ß1 or left untreated.

Figure 22 depicts the use of anti-B for immunofluorescence microscopic detection of elevated (2'-5') oligo A synthetase levels in lymphocytes from blood of patient with viral disease (middle panel). Right: control with normal serum: left: blood from healthy donor with anti-B stain. (Lymphocytes do not stain, only macrophages or granulocytes give unspecific background.

Detailed description of the invention

The present invention concerns human DNA encoding an enzyme having (2'-5') oligo A synthetase activity and having the nucleotide sequence set forth in Figure 7A. The DNA may also comprise the sequence of nucleotides 1-1322 set forth in Figure 7A and the overlapping sequence of nucleotides 901-1590 set forth in Figure 7B. The DNA of the present invention has the restriction enzyme sites set forth in Figure 9.

An enzyme having (2'-5') oligo A synthetase activity has the amino acid sequence set forth in Figure 7A. This enzyme comprises about 364 amino acids and has a molecular weight of about 41,500 daltons. another enzyme having (2'-5) oligo A synthetase activity comprises the sequence of amino acids 1-364 set forth in Figure 7A and the sequence of amino acids 290-400 set forth in Figure 7B. This enzyme comprises about 400 amino acids and has a molecular weight of about 46,000 daltons.

The present invention provides a 1.6 kb RNA having a nucleotide sequence complementary to the nucleotide sequence set forth in Figure 7A. Also provided is a 1.8 kb RNA comprising a nucleotide sequence complementary to the sequence of nucleotides 1-1322 set forth in Figure 7A and the sequence of nucleotides 901-1590 set forth in Figure 7B.

A transfer vector of the present invention comprises lambda-gt 11-E16 DNA of the present invention, and the lac Z gene, the DNA being fused in phase with the lac Z gene, so as to enable expression of the DNA in a suitable host cell. A microorganism may be transformed by the transfer vector. Escherichia coli is a suitable microorganism for the transformation.

A method of monitoring the response of a patient to an interferon comprising measuring the concentration of (2'-5') oligo A synthetase mRNA in cells or body fluids of the patient by hybridizing to the mRNA DNA complementary thereto. The mRNA may be the 1.6 kb or 1.8 kb RNA of the present invention.

A method for evaluating the response of cells and tissues to interferon comprises hybridizing RNA from cells or tissues exposed to interferon with cDNA complementary to the RNA, and determining the extent of hybridization. The RNA is extracted from cells or tissues which have been exposed to interferon, immobilized on a membrane filter and hybridized to labelled cDNA specific for interferon-induced mRNAs. The method may also comprise in situ hybridization of labelled cDNA to slices of tissues and then evaluating by microscopic examination autoradiography, or fluorescence. The cells or tissues analyzed may be of human or other animal origin.

A kit for carrying out a method for evaluating the response of cells and tissues to interferon contains a cDNA complementary to a sequence set forth in Figure 7A or 7B, reagents to carry out the hybridization tests for nick-translation with deoxyribonuclease I and [^{32}P]-gamma-dCTP, reagents for hybridization on nitrocellulose membranes, and reagents for RNA extraction from cells.

Also provided are antigenic peptides having amino acid sequences contained within the amino acid sequences set forth in Figure 7A and Figure 7B.

An antigenic peptide of the present invention has the amino acid sequence comprising the 17 C-terminal amino acids of the amino acid sequence set forth in Figure 7A and having the amino acid sequence: ARG-PRO-PRO-ALA-SER-SER-LEU-PRO-PHE-ILE-PRO-ALA-PRO-LEU-HIS-GLU-ALA: Another antigenic peptide has the amino acid sequence: GLU-LYS-TYR-LEU-ARG-ARG-GLN-LEU-THR-LYS-PRO-ARG-PRO-VAL-ILE-LEU-ASP-PRO-ALA-ASP.

Antibodies raised against the antigenic peptides of the present invention recognize and immunoprecipitate (2'-5') oligo A synthetase.

A method of monitoring interferon activity in a subject comprises measuring the amount of (2'-5') oligo A synthetase in a cell or body fluid of the subject at predetermined time intervals, determining the differences in the amount of said synthetase in the cell or body fluid of the subject within the different time intervals, and determining therefrom the amount of synthetase in the cell or body fluid of the subject and thereby the interferon activity of the subject. The amount of synthetase may be measured by contacting the synthetase with an antibody of the present invention so as to form a complex therewith and determining the amount of complex so formed.

A method of monitoring interferon activity may further comprise the extraction of (2'-5') oligo A synthetase from a cell or body fluid which has been exposed to interferon, labelling the extracted synthetase with an identifiable marker to form a labelled synthetase, contacting the labelled synthetase with an antibody of the present invention under suitable conditions so as to form a labelled synthetase-antibody complex, and detecting the marker in the complex, thereby detecting the synthetase. The marker may be ^{35}S-methionine.

A kit for carrying out the method of monitoring interferon activity comprises an antibody of the present invention, materials for extracting the synthetase, materials for labelling the synthetase, and materials for detecting the marker and determining the amount of synthetase.

The present invention also provides cloned DNA that specifically hybridizes to messenger RNAs which appear in human cells after exposure to interferon. The cloned cDNA may be specific for the (2'-5') oligo A synthetase mRNAs of 3.6, 1.8 and 1.6 kilobase. A cloned DNA of the present invention is specific for the mRNA of a 56,000 Mr-protein, which mRNA is 2 kilobase and which has the sequence defined in Figure 1.

A partial cDNA clone (E1) for the (2'-5') oligo A synthetase mRNA from human SV80 cells, was first obtained through its ability to select by hybridization a mRNA producing (2'-5') oligo A synthetase activity upon translation in Xenopus laevis oocytes (Merlin et al, 1983). The E1 cDNA insert (675 bp) hybridizes to 3 RNA species of 1.6, 1.8 and 3.6 kb which are coinduced by IFN in SV80 cells, accumulate for 12 hours and are found in the cytoplasmic polysomal fraction (Benech et al, 1985). Two other early transcripts (2.7 and 4 kb) appear in lesser amounts. Analysis of various types of human cells has shown that these RNAs are differentially expressed in a cell specific manner. In B lymphoblastoid cells (Namalva, Daudi) only the 1.8 kb RNA accumulates, while in amniotic WISH cells, in histiocytic lymphoma U937 cells and in HeLa cells, the 1.6 kb RNA is predominantly induced by IFN with some 3.6 kb RNA but little 1.8 kb RNA. In diploid fibroplasts FS11, in SV80 fibroplastoid cells and in the T cell line CEMT, all 3 stable RNAs are expressed (Benech et al, 1985). The type of (2'-5') oligo A synthetase RNA expressed does not depend on the species of IFN used (α, β or γ) but rather seems developmentally regulated in the cell.

The different (2'-5') oligo A synthetase transcripts appear to originate from a single gene (Benech et al, 1985). Restriction mapping showed 1) that the E1 cDNA corresponds to the 3' end of the 1.6 kb RNA, 2) that the 1.8 kb RNA has a different 3' end than the 1.6 kb RNA and ccntains an additional downstream exon, 3) that the 3.6 kb RNA has the same 3' end as the 1.8 kb RNA but is incompletely spliced. Hybridization-translation experiments using specific genomic DNA fragments also demonstrate that both the 1.8 and 1.6 kb RNAs actively code for (2'-5') oligo A synthetase (Benech et al, 1985).

cDNA clones for the 1.6 and 1.8 kb RNAs have been isolated and sequenced, which enabled the deduction of the amino acid sequences of two forms of the IFN-induced (2'-5') oligo A synthetase in human cells. The two proteins differ in their C-termini, which is hydrophobic in the 1.6 kb RNA product (E16) and acidic in the 1.8 kb RNA product (E18). A complete mapping of the (2'-5') oligo A synthetase gene shows that the 1.6 kb RNA is coded by 7 exons and the 1.8 kb RNA by 8 exons. The sequence of the presumed transcription initiation site and promoter region of the IFN-activated human (2'-5') oligo A synthetase gene shows a striking homology to the promoter region of the human IFN-β1 gene.

EXAMPLE 1

MEASURE OF (2'-5') OLIGO A SYNTHETASE mRNA by cDNA CLONES

A) Isolation of E-cDNA clones

Total RNA was prepared from 10^9 SV80 cells (SV40-transformed human fibroblasts) treated for 12 hours with 200 units per ml IFN-beta. The RNA was extracted by 3M LiCl -6M urea and purified by passage on oligo dT-cellulose. The 0.4 mg poly A^+-RNA obtained were fractionated in a preparation of gel electrophoresis apparatus in 1.5% agarose/6M urea 25mM sodium citrate pH 3.5. The 17-18S RNA fraction, was used to prepare cDNA as follows: 2 micrograms RNA were heated for 1 min at 90°C with 2 micrograms oligo $(dT)_{12-18}$ in 60 microliters water, cooled at 0°C, supplemented with salts to a final concentration of 50mM Tris-HCl pH 8.3, 10mM $MgCl_2$, 75mM KCl and incubatedd 5 min at 42°C before adding 1mM dithiothreitol, 1mM each dATP, dTGP, 0.5mM dCTP, 20 micro-Ci ^{32}P-dCTP (300 (Ci/mmol)), 4mM Na-pyrophosphate and 20 units reverse transcriptase in a final volume of 0.1ml. The mixture was incubated for 45 minutes at 42°C, the reaction was stopped with 10mM EDTA, 0.2% Na-dodecyl sulfate and the cDNA extracted with phenol-chloroform, treated with 0.3N NaOH for 2 hours at 52°C and neutralized. The cDNA was filtered on Sephadex G-75, ethanol precipitated, and tailed by dATP with terminal transferase. The synthesis of the second cDNA strand was primed with oligo (dT) and carried out as for the first strand for 2 hours at 42°C but without radioactive nucleotide and without pyrophosphate. To insure blunt ends the ds cDNA was incubated with E. coli DNA

polymerase I large fragment first in 20mM Tris-HCl pH 8, 75mM KCl, 5mM $MgCl_1$, 1mM dithiothreitol for 5 minutes at 37°C (trimming reaction) and then under the conditions of filling-in with ATP. The ds cDNA ws fractionated by sedimentation on a 5-20% sucrose gradient and the heaviest fractions were tailed with dCTP and annealed with equimolar amounts of Pst1-cut pBR322 plasmid DNA tailed with dCTP. About 7 ng DNA were mixed with 100 microliters of frozen, $CaCl_2$-treated, E. coli MM294. After 30 minutes at 0°C, and 5 minutes at 37°C, the bacteria were grown in 2ml of LB-broth for 2 hours at 37°C, and plated on LB-agar plates with 10 micrograms/ml tetracyline. About $1.4x10^5$ tetracycline-resistant, ampicillin-sensitive colonies were obtained per microgram recombinant plasmid DNA.

To identify the cDNA clone of the (2'-5') oligo A synthetase mRNA, a total of 3,000 plasmid DNA clones were screened by hybridization to RNA of IFN-treated SV80 cells, and the DNA-selected RNA was tested by injection into Xenopus laevis oocytes and a measure of the (2'-5') oligo A synthetase activity formed according to the method of Shulman and Revel (1980). Pools of plasmid DNA from 12 individual clones (3 micrograms DNA each; cut with Eco R1) were applied onto a 0.4cm diameter nitrocellulose filter and prehybridized for 2 hours at 37°C in 50% formamide, 2mM Pipes buffer pH 6.4, 0.75M NaCl, 1mM EDTA (buffer A). Three filters with pBR322 DNA and thirty filters of recombinant DNA pools were incubated together with 300 micrograms poly A^+-RNA (calculated to have a 10-fold excess of each insert cDNA over the presumed amount of (2'-5') oligo A synthetase mRNA, 0.09 micrograms or 0.03%) in 1ml buffer A for 20 hours at 37°C. The filters were washed twice at 37°C with buffer A, 4 times in 20mM Tris-HCl pH 7.5, 0.15M NaCl, 1mM EDTA, 0.5% Na dodecyl sulfate (once at 37°C and 3 times at 52°C) and then 4 times with 10mM Tris-HCl pH 7.5, 1mM EDTA (buffer C) at 52°C. Each filter was next washed individually in buffer C at 52°C and the RNA was eluted by heating 2 min at 96°C in 0.3ml buffer C with 40 micrograms rabbit liver tRNA per ml. After quick cooling the ethanol precipitation, the RNA was dissolved in 2 microliters water. Ten Xenopus laevis oocytes were microinjected with 0.7 microliters RNA and after 18 hours at 19°C, the oocytes were homogenized in their incubation medium (Shulman and Revel, 1980) and 0.15 ml of homogenate was mixed with poly (rI) (rC)-agarose beads.

The beads were incubated for 16 hours at 30°C with 2.5 mM (^{32}P)-alpha-ATP (0.3 Ci/mmol), 10 mM dithiothreitol, and 10 microliters of the liquid phase were incubated with 0.35 units bacterial alkaline phosphatase in 30 mM Tris-base for 60 minutes at 37°C. The digest was submitted to paper electrophoresis on Whatman 3MM paper at 3,000 V for 4 hours, and the spots corresponding to (2'-5') ApA and (2'-5') ApApA were cut and counted by scintillation. From the DNA-selected RNA, 1 microliter was used for in vitro translation in a reticulocyte lysate as described in Weissenbach et al. (1979), to measure the total mRNA activity of the sample.

The ratio of (2'-5') oligo A synthetase activity over total mRNA activity in the DNA-selected RNA samples was calculated for each filter. One filter with pool 174, out of the 250 pools of 12 individual plasmids, gave consistently a ratio about 10 times higher as other pools or as pBR322 DNA. The plasmid DNA of each individual clone of pool 174 was tested on separate filters and clone 174-3 was found to give consistently a 35-100 fold enrichment of the (2'-5') oligo A synthetase mRNA over total mRNA as compared to total RNA or pBR322 DNA-selected RNA (Table 1). Clone 174-3 was identified as the (2'-5') oligo A synthetase cDNA and designated E-cDNA. The structure and sequence of this cDNA is shown in Fig. 1. The E-cDNA clone contains the sequence for the 100 carboxy terminal amino acids of the enzyme and a 192 nucleotide-long untranslated region preceding the poly A-tail.

TABLE 1

IDENTIFICATION BY HYBRIDIZATION-TRANSLATION OF THE CLONE OF (2'-5') OLIGO A SYNTHETASE cDNA

E mRNA activity measured by oocyte injection (2) of induced RNA

	Expt. 1		Expt. 2		Expt. 3	
	(2'-5') oligo A cpm	(specific activity)*	(2'-5') oligo A cpm	(specific activity)*	(2'-5') oligo A cpm	(specific activity)*
Total poly A$^+$ -RNA	4050	(0.007)	3440	(0.004)	4900	(0.01)

RNA selected on: pBR filters	350 625	(0.05)	570 595	(0.03)	670 725	(0.02)	
plasmid pool 174	2320		-		-		
other pools	230** : 60		-		-		
Clones of pool 174: 1	560		700		995		
2	625		950	(0.04)	825		
3	6460	(4.78)	39,800	(2.7)	11,235	(0.75)	
4	600		1,820	(0.48)	1,030	(0.03)	
5	745		500	(0.19)	530		
6	985		475		630		
7	1270	(0.1)	365		605		
8	395		800	(0.02)	600		
9	490		100		1,030	(0.03)	
10	1465	(0.14)	290		1,155	(0.06)	
11	1860	(0.27)	365		735		
12	540		320		915		
No RNA	195		185		590		

*Specific activity ratio of (2'-5') oligo A synthesis in mRNA-infected oocytes to translation of same RNA in reticulocyte lysates.

** Average of 28 pools.

B) MEASURE OF INTERFERON-INDUCED (2'-5') OLIGO A SYNTHETASE mRNA BY HYBRIDIZATION OF E-cDNA.

Plasmid DNA of clone 174-3 (E-cDNA) can be used to detect the complementary RNA on electrophoretic blots of total cell RNA. Poly A^+-RNA is prepared from SV80 cells treated various times by 2000/ml interferon-beta and 7 micrograms RNA are denatured in 50% formamide, 6% formaldehyde, electrophoresed in 1.3% agarose with 6% formaldehyde and blotted onto nitrocllulose according to the procedures of Thomas (1980) and Fellous et al. (1982). The E-cDNA plasmid labeled by nick-translation with (^{32}P)-gamma-dCTP according to Merlin et al. (1983), is hybridized to the nitrocellulose blot.

In SV80 cells, three RNA species which are all coordinately induced by the interferon treatment, hybridize with E-cDNA (Fig. 2), a large RNA species of 3.6 kilobases and 2 smaller species of 1.85 and 1.65 kilobase. In non-treated SV80 cells, no E-specific RNA is found. The 3 RNA species appear at 4 hours, are maximum at 12 hours and decrease slowly thereafter. The RNAs are still clearly detected

at 24 hours after interferon. Additional RNA species seen only at 4 hours are most probably precursors of the more stable species. The same 3 RNA species are seen in human diploid fibroblasts treated by interferon. However, in cells of the hemopoietic lineage such as lymphoblastoid Namalva cells, only one main RNA species hybridizes to E-cDNA (Fig. 2) and corresponds to the 1.85 kilobase RNA species. The same RNA pattern is seen in other lymphoblastoid cells, in erythroid HL-60 and in promonocyte U937 cells.

The different E-specific RNA pattern in fibroblasts and lymphoid cells corresponds to different forms of the (2'-5') oligo A synthetase in these cells. Lymphoid cells contain an enzyme of molecular weight 30,000 daltons, while fibroblasts contain two forms of the enzyme of molecular weight 80,000 and 30,000 daltons, as reported by Revel et al. (1982). The small 1.85 kilobase mRNA is sufficiently long to code for the 30,000 Mr enzyme but not for the larger form, while the 3.6 kilobase E-mRNA codes for the 80,000 Mr form of the enzyme. All three E-specific RNA species hybridize to a single clone of human genomic DNA, and probably originate from a single gene, the 3.6 kilobase RNA having an additional interferon exon as compared to the 1.85 kilobase RNA.

Leucocyte interferon-alpha induces E.specific RNA as well as does fibroblast interferon-beta. The multiplicity of RNA species revealed by hybridization to E-cDNA suggests that different interferon species, which all induce (2'-5') oligo A synthetase, could induce different forms of the RNA and of the enzyme. Different interferon species can also vary in their efficacy for inducing E-mRNA.

RNA to be treated in the above hybridization assay to E-cDNA may be prepared from various cells in culture or from tissues taken from patients receiving interferon therapy or suffering from viral diseases or from disease in which an elevated (2'-5') oligo A synthetase was observed (Schattner et al., 1981). Rna may also be prepared from blood cells, such as leukocytes, obtained from peripheral blood. The electrophoretic blot can be replaced by a dot-hybridization method, in which RNA samples are directly applied to nitrocellulose in circles or rectangles of defined area, and the radioactive cDNA is hyb-

ridized to the nitrocellulose sheet. The radioactivity of each circle or rectangle is then measured by direct counting or by autoradiography followed by screening of the autoradiographic film.

An alternative method is to perform hybridization in situ on tissue slices obtained from biopsies of tissues exposed to interferon. This can be preferentially applied to brain biopsies in patients receiving interferon for a brain viral disease or tumor, in order to measure whether the brain has been exposed to interferon when the drug is given either by intrathecal injection or by systemic injection. The method may be applied to skin biopsies when the interferon treatment is given locally as an ointment for skin lesions. It is obvious that many other applications are possible. The tissue slices may be fixed and hybridized in situ to radioactive DNA, followed by an autoradiography with a sensitive photographic emulsion. The cDNA may also be labeled by flourescent nucleotides or by modified nucleotides which can bind fluorescent molecules, and the hybridization to the tissue slice can be monitored by fluorescent microscopy.

An increase in hybridization of the E-cDNA was compared to a proper control cell RNA or tissue sample, indicating that the cell or tissue has been exposed to interferon. The rapidity (4-24 hours) and sensitivity (1-200 units of interferon per ml) of the method makes it very useful to follow a treatment by external interferon, or formation of endogenous interferon in blood and tissue of patients.

EXAMPLE 2

CLONED cDNA FOR THE INTERFERON-INDUCED 56,000 Mr PROTEIN

A) Isolation of cloned C56-cDNA

The cloned cDNA was isolated from the library of recombinant plasmids described in Example 1. The principle of the method used was differential hybridization. Two duplicate sets of the 3,000 bacterial clones grown on nitrocellulose filters were hybridized either to (^{32}P)-cDNA from 17S-18S poly A^+-RNA of SV80 cells treated by interferon-beta (200 U per ml), or to (^{32}P)-cDNA from total poly A^+-RNA of non-treated SV80 cells. The radioactive cDNA were reverse transcribed from mRNA as in Example 1. About 40% of the bacterial clones

hybridized strongly to the "interferon-treated" cDNA probe and 8% gave a clear differential signal, hybridizing preferentially or uniquely to the "interferon-treated" cDNA as compared to the "non-treated" cDNA. The latter group of clones was then screened by hybridizing the plasmid DNA from each clone, labeled radioactively by nick-translation, to electrophoretic blots of RNA from interferon-treated SV80 cells and from non-treated cells. By this criterion, 1-2% of the original 3,000 bacterial clones were found to contain a plasmid cDNA clone corresponding to an interferon-induced mRNA. One of these plasmid cDNA clones, designated C56, showed a particularly strong differential hybridization. This C56 DNA hybridizes to an 18S RNA present in interferon-treated cells but completely absent from control cell RNA (Fig. 3). In comparison, HLA-A,B,C mRNA which is increased 5-fold in SV80 cells after interferon-treatment (Fellous et al., 1982), appears much less induced than C56 mRNA and under the experimental conditions of Fig. 3, gives a clear signal also with "non-treated" RNA.

The mRNA selected by hybridization to C56 cDNA immobilized on nitrocellulose filters, followed by elution from the films (as in Example 1) was translated in a reticulocyte lysate cell-free system and the (^{35}S) -methionine-labeled translation products were analyzed by polyacrylamide gel electrophoresis in Na-dodecyl sulfate according to the method described in Weissenbach et al. (1979) adapted from Laemle (1970). The C56 cDNA-selected RNA is translated into a 56,000-Mr protein. The sequence of the C56 cDNA permits one skilled in the art to deduce 65 amino acids of the carboxy terminal sequence of the 56,000 Mr protein (Fig. 4).

Hybridization of the C56 cDNA to RNA extracted from SV80 cells treated various times by interferon-beta (200 U per ml), shows that the C56 mRNA starts to appear at 1 hour after interferon addition (Fig. 5). The C56 RNA reaches its maximum after 4 hours, but is still detectable, although reduced, at 24 hours. Induction of C56 mRNA was also demonstrated in dipoid fibroblasts, and in lymphoblastoid cells. Induction was proportional to the concentration of interferon between 10 and 200 units per ml. C56 mRNA was also induced by interferons alpha and gamma, although the latter was less efficient.

The absence of this mRNA in non-treated cells and its strong and rapid increase after interferon addition make the C56 cDNA an excellent probe to evaluate the response of cells to interferon. The techniques described for E-cDNA in Example 1, can be similarly applied to the C56 cDNA.

The availability of a number of cDNA corresponding to mRNA induced by interferon offers new perspectives. In particular, interferon is needed at 100-fold lower concentrations to induce HLA-A,B,C mRNA than to induced E-mRNA or C56 mRNA (Wallach et al. (1982)); on the other hand, some subspecies of interferon-alpha, such as alpha-d can induce E-mRNA when a concentration 100 times lower than those needed to induce HLA-A,B,C mRNA. A comparison of the hybridization of different cloned cDNAs to the same RNA sample, can indicate what type of interferon is involved. Thus, more information can be derived from the comparison of different cDNA than from the use of only one cDNA probe.

EXAMPLE 3

A KIT FOR THE MEASURE OF INTERFERON-INDUCED mRNAs

The Kit would provide the cloned cDNA specific for the mRNA of the (2'-5') oligo A synthetase and for the mRNA of the 56,000 Mr protein, described herein, as well as reagents to carry out the hybridization tests: comprising reagents for nick-translation with deoxy-ribonuclease I and (^{32}P)-gamma-dCTP, reagents for hybridization on nitrocellulose membranes, and reagents for RNA extraction from the cells.

EXAMPLE 4

SEQUENCE OF cDNA FOR THE 1.6 KB (2'-5') OLIGO A SYNTHETASE mRNA

The partial E1 cDNA clone (Merlin et al, 1983), shown to be the 3' end of the 1.6 kb (2'-5') oligo A synthetase by IFN in human cells (Benech et al, 1985) was used to screen a lambda gt10 cDNA library from SV80 cell RNA (Wolf and Rotter, 1985). By restriction mapping, clone lambda gt10 9-2 was found to contain the E1 cDNA at the 3' end of a 1.32 kb EcoR1 insert (Fig. 6A) which was subcloned in pBR (9-21 cDNA). Sequencing was carried out as outlined in Fig. 6A

and confimed that the 9-21 cDNA contains the C-terminus and 3'untranslated sequence previously reported for the E1 cDNA (Merlin et al, 1983). The 9-21 cDNA sequence (Fig. 7) predicts an open reading frame of 364 aminoacids starting at an ATGATG sequence. A computer program based on the 3-base periodicity of protein-coding sequences (Trifonov, 1984) indicated that the only compatible reading frame is the one starting from this ATGATG. It is possible that translation initiates at the second ATG in this site, since it is the only one preceded by an A at -3 and having homology with the consensus translation initiation sequence (Kozak, 1984).

The enzyme thus coded by the 1.6 kb (2'-5') oligo A synthetase RNA would have a molecular weight of 41,700, in good agreement with the apparent 38,000-Mr protein seen by SDS-polyacrylamide gel electrophoresis of the in vitro translation product of RNA hybridized to E1 cDNA (Merlin et al, 1983). The C-terminal heptadecapeptide predicted by the open reading frame, was synthesized chemically and used to immunize rabbits. The antiserum obtained (C in Fig.12) precipitates specifically a protein migrating at 38,000-Mr in SDS gel electrophoresis from 35S-methionine labeled extracts of cells treated by IFN which is absent from untreated cells. Two experiments confirmed that this protein has (2'-5') oligo A synthetase activity: it was removed from the extracts by passage through a poly (rI)(rC) agarose column, and the supernatant remaining after immunoprecipitation was depleted of a large part of the enzymatic activity.

EXAMPLE 5

SEQUENCE OF cDNA FOR THE 1.8 KB (2'-5') OLIGO A SYNTHETASE mRNA

A genomic DNA fragment corresponding to the additional exon of the 1.8 kb RNA (Benech et al, 1985; see Fig. 9) was used as probe to isolate a E18 cDNA clone, 48-1, from the same lambda gt10 cDNA library of SV80 RNA. The restriction map of the E18 cDNA clone (Fig. 6B) confirmed that its 5' end is part of the E16 cDNA but that its 3' end differs. Sequencing (Fig. 7) revealed that the junction is at nucleotide 1071 of the E16 9-21 cDNA clone, the last 247 nucleotide of E16 being replaced by a 515 nucleotide-long sequence terminated by a different polyadenylation site. This difference accounts for the 0.2 kb difference in size between the two

mRNAs seen on Northern blots. The 5' portion of the E18 cDNA shows no base change from the sequence of the E16 cDNA, but is incomplete. The gene mapping described below, indicates that both 1.6 and 1.8 kb mRNAs have the same 5' end.

The 3' region of the E18 cDNA which diverges from the E16 sequence, contains an open reading frame ending after 54 codons. This reading frame, which leaves a 350 nucleotides-long untranslated region, was confirmed by the computer program based on the 3 base periodicity of protein-coding sequences (Trifonov, 1984). An alternate longer open reading frame would not be in the same computed phrase as the 5' portion common with the E16 cDNA. A hydropathy plot (Kyte and Doolittle, 1982) on the predicted C-termini of the 1.6 and 1.8 kb mRNA protein products, indicates a striking difference between the two forms of the (2'-5') oligo A synthetase (Fig. 8). The C-terminus of the E16 protein is very hydrophobic, while that of the E18 protein is hydrophilic and contains two acidic regions (Asp-Asp-Glu-Thr-Asp-Asp and Glu-Glu-Asp) (Fig. 7). Futhermore, a possible glycosylation site is present in the C-terminus of the E18 product (Fig. 7).

The 9-21 cDNA was subcloned in λgtll so as to fuse the coding frame in phase with the lac Z gene. Extracts of the E.coli lysogen containing this phase, showed clearly (2'-5') oligo A synthetase activity after binding to poly(rI)(rC) agarose, while no activity was found when the 9-21 cDNA has been fused in the opposite orientation (Fig.13). This expression in E.coli demonstrates that the cDNA indeed corresponds to the structural gene coding for the ds RNA activated (2'-5') oligo A synthetase and that the protein of about 40 kd coded by the IFN induced RNA is the enzyme itself, and not a regulatory factor. This protein does not seem to require post-translational modifications to exhibit enzymatic activity.

The transformed cell containing the 9-21 cDNA has been designated Escherichia coli lambda-gtll-E16 and deposited under Accession No. I496 with the Collection National Cultures de Micro-organismes, Institut Pasteur, 25 rue du Docteur Roux, 75724-Paris-Cedex 15, France. This deposit was made pursuant to the Budapest Treaty On the

International Recognition Of The Deposit Of Microorganisms For The Purposes Of Patent Procedure.

EXAMPLE 6

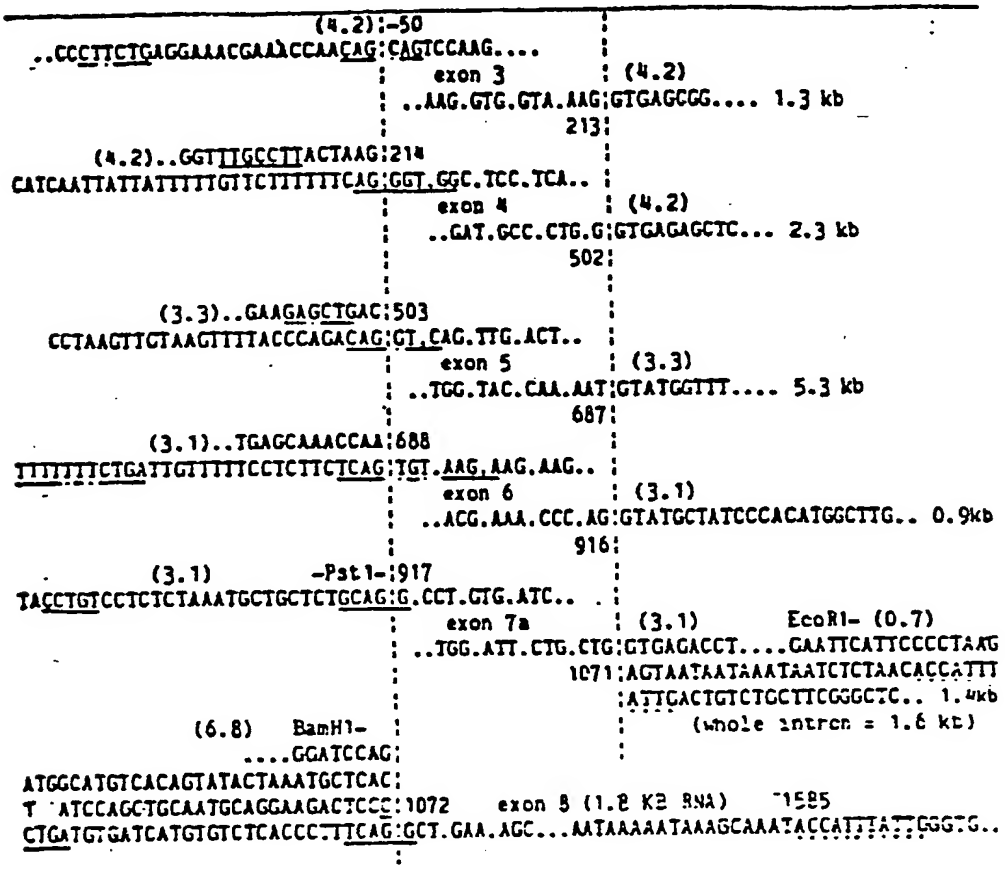
ORGANISATION OF THE HUMAN (2'-5') OLIGO A SYNTHETASE GENE

Three overlapping genomic clones were isolated using the E1 cDNA as probe (Benech et al, 1985), one from a library of partial EcoR1 digest of human blood cell DNA (Mory et al, 1981) and two from a library of partial Alu1 and Hae3 digest of embryonic human DNA (Maniatis et al, 1978). The genomic clones represent about 29 kb of human DNA and no evidence for more than one E gene was found while screening the libraries. Southern blots of genomic DNA are consistent with the existence of a single gene (Fig. 9). By Northern blot analysis using genomic DNA fragments as probes, by S1 nuclease mapping and by sequencing, the E16 cDNA 9-21 was shown to correspond to five exons on the gene (Fig. 9). The ATGATG sequence is found in exon 3, while the termination codon and 3' untranslated region with the polyadenylation site of the 1.6 kb RNA are found in exon 7. The structure of the more 5' exons 1 and 2 is described below. The sequences of the intron-exon boundaries were determined (Table 2) and follow the CAG and GT rule for the splice acceptor and donor sites (Breathnach and Chambon, 1981). A sequence CTGAC/T is commonly found not far from the splice acceptor, as reviewed recently by Keller (1984). It is notable that the CTGAC/T region shows base complementarity to the sequence of the intron/exon 3' boundary (acceptor site; Table 2), in addition to the complementarity of the intron donor site with the CTGAC sequence pointed out by Keller (1984) as playing a role in the lariat model.

The sequences of the 5 exons containing the coding region of the (2'-5') oligo A synthetase produced by the 1.6 kb mRNA, would indicate that the enzyme is composed of domains with differing amino-acid compositions (Table 3). The first exonic domain (60 amino acids) is rich in aspartic acid, in the second (aminoacids 61 to 156) arginine is predominant, the two next exons (aminoacids 157 to 218 and 219 to 295) are lysine rich, and the C-terminus of the E16 product (296 to 364) is very rich in proline and alanine.

TABLE 2

EXON-INTRON BOUNDARIES IN THE HUMAN (2'-5') OLIGO A SYNTHETASE GENE



For exon numbering see Fig. 7 and 9. The self-complementary regions between the CTGAT/C, or CTTAC, CTGTC (Keller, 1984) and splice acceptor CAG are underlined. The polyadenylation sites with a conserved undecanucleotide of the 1.6 and 1.8 kb RNAs (see Fig. 7) are underscored by dots. The numbers in parentheses are the size of the Eco RI genomic fragments carrying the introns or exons (see Fig. 9). The start and end of each exon is numbered as in the 9-21 E cDNA of Fig. 7.

TABLE 3

EXONIC DOMAINS OF THE E16 AND E18 (2'-5') OLIGO A SYNTHETASES

AA	1-60 (60)	61-156 (96)	157-218 (62)	219-295 (77)	296-346 (51)	E16 C-term. 347-364 (18)	E18 C-term. 347-400 (54)
ALA	2 (3.3)	7 (7.3)	0 (0.0)	3 (3.9)	4 (7.8)	3 (16.7)	4 (7.4)
ARG	4 (6.7)	10 (10.4)	3 (4.8)	5 (6.5)	1 (2.0)	1 (5.6)	2 (3.7)
ASN	1 (1.7)	2 (2.1)	2 (3.2)	3 (3.9)	3 (5.9)	0 (0.0)	1 (1.9)
ASP	6 (10.0)	5 (5.2)	2 (3.2)	1 (1.3)	4 (7.8)	0 (0.0)	5 (9.3)
CYS	4 (6.7)	1 (1.0)	2 (3.2)	2 (2.6)	1 (2.0)	0 (0.0)	1 (1.9)
GLN	1 (1.7)	7 (7.3)	6 (9.7)	5 (6.5)	2 (3.9)	0 (0.0)	3 (5.6)
GLU	2 (3.3)	7 (7.3)	5 (8.1)	4 (5.2)	2 (3.9)	1 (5.6)	5 (9.3)
GLY	2 (3.3)	9 (9.4)	3 (4.8)	3 (3.9)	6 (11.8)	0 (0.0)	2 (3.7)
HIS	1 (1.7)	0 (0.0)	1 (1.6)	1 (1.3)	0 (0.0)	1 (5.6)	3 (5.6)
ILE	5 (8.3)	2 (2.1)	3 (4.8)	4 (5.2)	2 (3.9)	1 (5.6)	2 (3.7)
LEU	5 (8.3)	13 (13.5)	8 (12.9)	10 (13.0)	6 (11.8)	2 (11.1)	2 (3.7)
LYS	5 (8.3)	2 (2.1)	7 (11.3)	9 (11.7)	2 (3.9)	0 (0.0)	1 (1.9)
MET	3 (5.0)	0 (0.0)	0 (0.0)	1 (1.3)	0 (0.0)	0 (0.0)	0 (0.0)
PHE	4 (6.7)	7 (7.3)	3 (4.8)	3 (3.9)	1 (2.0)	1 (5.6)	1 (1.9)
PRO	3 (5.0)	4 (4.2)	3 (4.8)	4 (5.2)	6 (11.8)	5 (27.8)	4 (7.4)
SER	4 (6.7)	8 (8.3)	3 (4.8)	1 (1.3)	3 (5.9)	2 (11.1)	5 (9.3)
THR	2 (3.3)	4 (4.2)	5 (8.1)	6 (7.8)	1 (2.0)	0 (0.0)	8 (14.8)
TRP	0 (0.0)	1 (1.0)	1 (1.6)	2 (2.6)	4 (7.8)	0 (0.0)	1 (1.9)
TYR	2 (3.3)	0 (0.0)	3 (4.8)	7 (9.1)	1 (2.0)	0 (0.0)	4 (7.4)
VAL	4 (6.7)	7 (7.3)	2 (3.2)	3 (3.9)	2 (3.9)	1 (5.6)	0 (0.0)

Although the E18 cDNA 48-1 is incomplete, we found that exons 1-6 (Fig. 9) hybridize to the 1.8 kb mRNA as well as to the 1.6 kb mRNA on Northern blots. The structure of the two RNAs is most likely identical up to exon 7. The additional splicing from the middle of exon 7 to exon 8 characterizing the E18 cDNA, was confirmed by sequencing tehse intron-exon boundaries in the genomic DNA clone (Table 2). The truncated exon 7a present in the E18 cDNA is followed by a 1.6 kb intron containing the polyadenylation site of the 1.6 kb RNA. Exon 8 begins 98 bp downstream from the unique BamH1 site of the gene (Table 2, Fig. 9). The genomic exon 8 ends by the polyadenylation site of the 1.8 kb RNA, characterized by a tandem repeat of the AATAAA signal. Although exon 7 and 8 have no homology, a conserved undecanucleotide ACCATTTATTG, in which the third cytidine is polyadenylated, is present at the end of both exons (Table 2). As pointed our previously (Benech et al, 1985), a hairpin-loop structure can be formed in both cases between this conserved and undecanucleotide and the AATAAA region; such structures may participate in the cell-specific mechanism which determines whether cleavage and polyadenylation of the transcripts occur

at the end of exon 7 or at the end of exon 8.

Based on the above gene mapping, the enzyme coded for by the 1.8 kb mRNA should be identical to the E16 product in the first 346 amino-acids, which are followed by a specific 54 aminoacid-long region, rich in aspartic, glutamic acid and threonine. The 400 aminoacid-long E 18 enzyme would have a molecular weight of 46,000.

EXAMPLE 7

TWO FORMS OF THE HUMAN (2'-5') OLIGO A SYNTHETASE PRODUCED BY ALTERNATIVE SPLICING OF THE SAME GENE

Northern blot analysis of SV80 RNAs revealed that 3 species of RNA (1.6, 1.8 and 3.6 kb) hybridizing to E1 cDNA accumulate in cells up to 12 hours after exposure to IFN (Merlin et al, 1983). Additional unstable transcripts were also seen. The relationship between these RNAs was investigated by transcript mapping on genomic DNA clones. In two human genomic libraries, the E1 cDNA identified only one series of overlapping genomic DNA clones which represent 29 kb of human DNA (Fig. 9A) and were found to contain an apparently unique (2'-5') oligo A synthetase gene (Benech et al, 1985a). By S1 nuclease analysis and partial gene sequencing, the 9-21 (E1) cDNA was found to correspond to 5 exons (numbered 3-7 on Fig. 9A and in the sequence of Fig.7). The 3' end and polyadenylation site of this cDNA was indentified at the end of exon 7 (Fig. 9). However, hybridization of further downstream genomic DNA fragments to Northern blots of SV80 RNA, revealed (Benech et al, 1985) that only the 1.6 kb RNA ended at the polyadenylation site in exon 7, while both the 1.8 and 3.6 kb RNAs hybridized to an additional exon located 1.6 kb downstream and which ends also by a polyadenylation site (exon 8, Fig. 9). Thus the 9-21 (E1) cDNA represents the 1.6 kb RNA and was renamed E16 cDNA. It was further found that the 3' half of exon 7 does not hybridize to the 1.8 kb RNA indicating that the transcript is formed by a splicing event from the middle of exon 7 to exon 8. All the 5' upstream exons hybridized to both 1.6 and 1.8 kb RNAs, indicating that the 2 RNAs differ only in their 3' ends. This was confirmed by the isolation

from the SV80 λgt10 cDNA library of a cDNA clone for the 1.8 kb RNA (clone 48-1 or E18 cDNA, Fig. 7B), which demonstrated the differential splicing and ended at the polyadenylation site of exon 8 (Fig. 4). A similar cDNA clone was found in a Daudi cDNA library by Saunders and Williams (1984). The E18 sequence locks the last 247 nucleotides of E16 which are replaced by 515 nucleotides accounting for the difference in size between the 1.6 and 1.8 kb RNAs.

The 1.8 kb RNA would thus code for a 46,000-Mr protein (E18) which differs from the E16 protein in its C-terminus. Like the E16 protein, the E18 product has ds RNA binding and (2'-5') oligo A synthetase activity as shown by translation of mRNA selected by hybridization to E18-specific DNA fragments (Benech et al, 1985). This suggests that the first 346 aminoacids common to the 2 proteins contain the catalytic sites. Examining the exon composition this common part appears composed of a N-terminal acidic domain, followed by three basic regions. The last 18 residues of the E16 protein form a very hydrophobic domain, which is replaced in E18 by a longer hydrophilic and acidic region which also contains a potential glycosylation site. This difference between the 2 enzymes may determine their ability to dimerize, or interact with other proteins and cellular structures. For example, E16 may bind to membranes while E18 may interact with basic proteins in ribosomes or in the nucleus.

Two forms of the (2'-5') oligo A synthetase were found by gel filtration in extracts of IFN-treated human cells (Revel et al, 1982): a 30-40 Kd enzyme which could correspond to a monomeric form of the E16 or E18 proteins, and a 60-80 Kd enzyme which remains to be identified. The 3.6 kb RNA does not seem to code for a large enzyme since transcript mapping showed that this RNA contains intronic regions (e.g. between exon 7 and 8) which are removed from the 1.8 kb RNA and have no open reading frame. We also failed to see large E mRNA in oocyte translations. A 80 Kd protein in SDS was reported in purified human (HeLa) synthetase (Yand et al, 1981) but its enzymatic activity was not demonstrated. In enzyme purified from Namalva and CML cells (Revel et al, 1981b) we could

detect a 40 Kd band in SDS. Thus it remains possible that the 60-80 Kd enzyme form is a dimer of the 40 Kd protein. The human synthetase may differ from that in mouse cells where a large 3.8 kb RNA was seen under denaturing conditions which codes for a 80 Kd enzyme (mainly cytoplasmic), in addition to a 1.5 kb RNA coding for a 30 Kd enzyme (mainly nuclear) (St. Laurent et al, 1983). The human E cDNA detects a 3,8-4 kb and a 1.6-1.7 kb RNA in mouse cells, the large RNA hybridizing to E18-specific DNA (Mallucci et al, 1985). It is possible that in human cells the large RNA is further processed into 1.8 kb RNA, which has not been seen in mouse cells. Shulman et al (1984) have used the fact that the bulk of the (2'-5') oligo A synthetase in human cells behaves as a smaller protein than in mouse cells to map the human synthetase gene to chromosome 11 in human rodent-hybrid cells. Antisera specific to E16 and E18 will help to elucidate the relationship between these proteins and the two forms of the native enzyme seen in human cells.

EXAMPLE 8

CELL-SPECIFIC EXPRESSION OF THE TWO (2'-5') OLIGO A SYNTHETASE mRNAs

RNA from a number of human cell lines have been examined in Northern blots with the E cDNA probes (Merlin et al, 1983; Benech et al, 1985). Table 4 shows that human cells can be grouped in 3 classes according to the predominant E mRNA species induced by IFN. Lymphoblastoid B cell lines from Burkitt lymphomas have mainly the 1.8 kb RNA. Instead, several cell lines have the 1.6 and 3.6 kb RNA but little 1.8 kb RNA. If the 3.6 kb RNA is a partially spliced precursor of the 1.8 kb RNA, these cells may have an inhibition in the processing of the 3.6 kb RNA. T-lymphocyte lines (CEMT from an acute leukemia and Gash from hairy cell leukemia) contain like fibroplastic cells, all 3 E RNA species. The E18 polyadenylation (pA) site seems, therefore, to be used in all human cells to produce either 3.6 or 1.8 kb RNA. The E16 pA site seems not to be used in B lymphoblastoid cells. A conserved undecanucleotide present in both E16 and E18 pA sites (Fig. 9B) can form a hairpin-loop with the AATAAA signal and could have a role in site selec-

tion (Benech et al, 1985). E18 has a tandem repeat of the AATAAA signal (Fig. 9B) and could be a stronger pA site. Transcripts ending at the E18 pA site accumulate earlier after IFN addition than the 1.6 kb RNA (Benech et al, 1985).

TABLE 4

PREDOMINANT (2'-5') OLIGO A SYNTHETASE RNA SPECIES

3.6 kb		3.6 kb
	1.8 kb	1.8 kb
1.6 kb		1.6 kb
Histiocytic lymphoma U937	B lymphoblastoid Burkitt lymphoma: - Daudi - Namalva - Raji	Fibroblastic: -SV80 -FS11
Amniotic Wish		
Cervix Ca HeLa		T cells: -CEMT
Raji x HeLa hybrids		Hairy cell-leuk.: -Gash

The type of synthetase predominantly made may vary in different human cells. We found no correlation between the cytoplasmic or nuclear localization of the synthetase and the type of RNA present in the cells. However, Namalava cells seemed to have mainly the 30-40 Kd enzyme upon gel filtration while HeLa and SV80 cells had also the 60-80 Kd form (Revel et al, 1982).

EXAMPLE 9

PROMOTER REGION OF THE (2'-5') OLIGO A SYNTHETASE GENE

The Sph1-Sph1 fragment of 0.85 kb (Fig.10) from the genomic 4.2 kb EcoR1 fragment (Fig. 9) which contains part of exon 3 of the E16 cDNA 9-21 clone, hybridized in Northern blots with the 1.6, 1.8, 2.7 and 3.6 kb RNAs. However, upstream regions did not. Several

experiments allowed to localize the RNA transcriptional start in its fragment. S1 nuclease analysis first showed that exon 3 starts about 50 nucleotides upstream of the end of the 9-21 cDNA. A primer extension experiment using an oligonucleotide from the end of the 9-21 cDNA, indicated that the 5' end of the mRNA is about 230 nucleotides from the 5' end of this cDNA. RNA hybridization with riboprobes produced in SP6 (Green et al, 1983) and RNase digestion indicated two exons of 70 and 110 nucleotides preceding exon 3. By S1 nuclease analysis using a probe labeled at the unique Hpa1 site (Fig. 9), the 5' end of the mRNA was finally located 17 nucleotides upstream from the Hpa1 site. The sequence of this region is shown in Fig. 6. The location of the transcription initiation site 17 residues before the Hpa1 site, is supported by the presence of a TATAA box at position -30. A striking feature of the upstream sequences, is the high purine content (69.6%) mostly adenine (58.9%). Run of a homology materix with other known promoter upstream sequences, revealed a surprising homology with the human IFN promoters, in particular with the sequence of the IFN-β1 gene promoter (Degrave et al, 1981). The purine-rich region from -75 to -85 of the IFN-β1 promoter, which contains the essential transcription signal described by Zinn et al (1983), shows 90% homology with the region of the presumed promoter of the (2'-5') oligo A synthetase just upstream of the TATAA box (-40 to -50) (Fig. 11). This purine-rich signal is repeated in the IFN-β1 promoter in the segment between the TATAA box and the cap site; in this region, which may also have regulatory functions (Nir et al, 1984) the homology between the IFN-β1 gene and the (2'-5') oligo A synthetase gene is high. In contrast, search for homology with promoters of other genes, such as HLA genes (Malissen et al, 1982; Schamboeck et al, 1983) and the metallothionein II gene (Karin and Richards, 1982) which are activated by IFNs (Fellous et al, 1982; Rosa et al, 1983b; Friedman et al, 1984) showed no apparent sequence relationship in this region of the (2'-5') oligo A synthetase gene promoter. No significant homology was also seen with the body of the IFN-β1 gene.

The 5' untranslated leader of the (2'-5') oligo A synthetase mRNA (exon 1, 2 and part of exon 3) contains two short introns whose

position was tentatively determined by S1 analysis as shown in Fig. 6. The entire human (2'-5') oligo A synthetase gene is about 13 kb (Fig. 9) and the sum of the exons agrees with the observed sizes of the mRNAs.

EXAMPLE 10

LAMBDA GT10 cDNA CLONES OF THE (2'-5') OLIGO A SYNTHETASE

A λgt10 cDNA library prepared from poly A+ RNA of human SV80 cells (Wolf and Rotter, 1985) was screened using as probe the Pst1-Pst1 insert of the E1 cDNA plasmid described previously (Merlin et al, 1983). The insert corresponding to the 3' end of the 1.6 kb E RNA (Benech et al, 1985), was purified by agarose gel electrophoresis and nick-translated (Rigby et al, 1977). Plaques were repeatedly picked from 9cm plates (10^5 phages), and small scale λDNA preparations were analyzed by restriction mapping by routine procedures (Maniatis et al, 1982). Fifteen λ gt10 cDNA clones containing the E1 cDNA fragment were isolated and phages 9-2 and 5-2 with the longest inserts were cut by EcoR1 and the inserts subcloned in pBR322 to obtain E16 cDNA clones 9-21 and 5-21 of Fig. 1A. The same library was rescreened with a human genomic Pst1-Pst1 0.9 kb fragment from phage λchE1 (Benech et al, 1985), a fragment which specifically hybridizes to the 1.8 kb RNA. We thereby isolated λgt10 cDNA clone 48-1 of Fig. 1B, along with another cDNA clone representing a partially spliced E RNA. Sequencing was carried out according to Maxam and Gilbert (1980). Restriction enzymes were from New England Biolabs and Boehringer. Homology matrix and hydropathy plot computer programs of Pustell and Kafatos (1982a,b) were run on IBM PC. Three base periodicity to locate protein coding frames was computed according to Trifonov (1984).

EXAMPLE 11

GENOMIC DNA CLONES CONTAINING THE (2'-5') OLIGO A SYNTHETASE GENE

Three overlapping genomic clones were isolated as previously described (Benech et al, 1985): λchE1 from a partial EcoR1-cut

DNA library (Mory et al, 1981) and λchE2 and E3 from a partial Alu1/Hae 3 DNA library (Maniatis et al, 1978). The genomic EcoR1 fragments of these phages were subcloned in pBR322. Exon mapping was done 1) by Southern blot hybridization of restriction digests from subcloned genomic fragments to various cDNA probes, 2) by hybridization of genomic DNA restriction fragments to Northern blots of poly A+ RNA from IFN-treated and untreated cells as described (Benech et al, 1985) and 3) by sequencing of intron-exon boundaries in comparison to cDNA.

The internal Sph1-Sph1 0.87 kb segment of the genomic 4.2 kb EcoR1 fragment containing the 5' end of the mRNA, was subcloned in the Sph1 site of pBR322 before sequencing. Primer extensions using synthetic oligodeoxyribonucleotides of 18-20 bases complementary to the mRNA (gift of Dr. D. Segev, InterYeda) were done as before (Rosa et al, 1983a). Riboprobes synthesis after subcloning in the SP6 vector were carried out according to instructions of Promega Biotec. DNA from Daudi lymphoblastoid cells and from FS11 foreskin fibroblasts was prepared according to Wigler et al (1979) and Southern blot analysis was done on Gene-Screen Plus nylon fiber sheets using hybridization procedure B recommended by the manufacturer (New England Nuclear).

EXAMPLE 12

QUICK CELL BLOT ASSAY OF (2'-5') OLIGO A SYNTHETASE RNAs FOR THE CLINICAL MONITORING OF IFN ACTION

The usefulness of measuring the (2'-5') oligo A synthetase has been shown in human peripheral blood mononuclear cells (PBMC) to monitor the response of patients to IFN-β (Schattner et al, 1981a) and IFN-β i.m. injections (Schoenfeld et al, 1984). Since the enzyme level of PBMC in normal individuals is rather constant, this assay has allowed to diagnose viral infections evidence by an increase in the enzyme in the PBMC and granulocytes (Schattner et al, 1981b, 1984; Schoenfeld et al, 1985). Decrease in the enzyme characterize acute leukemias with numerous blust cells (Wallach et al, 1982; Schattner et al, 1982). This technique has also been pioneered

by Williams et all (1981) and is now in wide use.

Synthetase E is strongly induced in cells treated by all three types of IFNs, alpha, beta and gamma, and its increase is a good marker of IFN activity (Wallach et al., 1982). It is therefore possible to use measurements of E levels to determine whether cells in vitro or in vivo have been exposed to IFN and respond to it. This measurement may be used as an assay for IFN in unkown solutions, by exposing cells to said solutions and determining the icnrease in E levels (Revel et al., U.S. Patent No. 4,302,533). The measurement may also be used to establish whether IFN is produced in increased amounts in whole organisms including man.

The (2'-5') oligo A synthetase increases during differentiation of hematopoietic cells as a result od autocrine secretion of IFN-beta (Yarden et al., 1984). Another important application of E measurements is in the monitoring of patients under IFN therapy. Besides clinical changes, it is possible to establish that the patients respond to IFN by measuring the PBMC E level which increases 5-10 fold during systemic IFN-alpha as well as beta treatment (Schattner et al., 1981a; Schoenfeld et al., 1984). It is clear that assay of other IFN-induced activities or molecules may be used as well as the assay of the E enzyme, but this method has been the most widely used (Williams et al., Borden).

Now the assay of E RNA in human PBMC is used for the same purpose. A quick cell blot (Cheley and Anderson, 1984) using the 9-21 E cDNA as probe was developed for PMBC (Fig. 14). Oligonucleotides derived from the E cDNA may also be used as probes. The effect of 10 U/ml IFN can easily be detected by this method (Fig. 15). Positive signals were obtained in a patient treated by 10^7 units/day of IFN-alpha-c.

EXAMPLE 13

OBTENTION OF ANTIBODIES TO (2'-5') OLIGO A SYNTHETASE

Two peptide sequences were chosen from the total aminoacid sequences

of E16 and E18, to serve as antigens for the induction of antibodies against the native (2'-5') oligo A synthetase activity molecule.

Peptide B:

GLU LYS TYR LEU ARG ARG GLN LEU THR LYS PRO ARG PRO VAL ILE LEU ASP PRO ALA ASP

comprises aminoacids 284 to 303 common to both E18 and E16 sequences.

Peptide C:

ARG PRO PRO ALA SER SER LEU PRO PHE ILE PRO ALA PRO LEU HIS GLU ALA

comprises the C terminus of E16 (residues 348 to 364). Both peptides were synthesized by the solid-phase peptide synthesis method of Barany and Merrifield (1980). After purification on Sephadex G25 columns in 2M acetic acid, the peptides were linked to Keyhole Limpet Hemocyanin (Calbiochem). Esterification of the NH_2 -terminal arginine of peptide C with p-aminophenylacetic acid allowed to covalently link the peptide to the carrier protein through its amino-terminus (Spirer et al, 1977). Peptide B was coupled to the carrier protein by ethylene diamine carbodiimide (Hoare and Koshland, 1967).

Rabbits were injected subcutaneously with 1 mg carrier-coupled peptide (equivalent to 0.2 mg pure peptide) which was emulsified in complete Freund's adjuvant. Rabbits were boosted twice at two weeks intervals with 0.5 mg of carrier-coupled peptide in incomplete adjuvant, and were continued until maximal antibody response. The titer of antibodies in the rabbit sera were measured in enzyme-linked immunosorbent assays (Green et al, 1982) using the carrier-free peptides.

EXAMPLE 14

USE OF ANTI-(2'-5') OLIGO A SYNTHETASE ACTIVITY PEPTIDE ANTIBODIES TO DETECT THE ENZYME

Extracts of IFN-treated human cell cultures

The fibroblastoid cell line SV80 and the amniotic cell line Wish were grown to confluent monolayers on plastic dishes and the Daudi cell line was grown in suspension to 1.5×10^6 cells/ml. Cultures were treated for 16-24 hours with rIFN-β1, 500 U/ml. The human rIFN-β1

was produced by genetically engineered CHO cells and purified to homogeneity by monoclonal antibody affinity chromatography (Chernajovsky et al, 1984).

Cells were washed twice with phosphate buffered saline (PBS) at 4°C and lysed in the cold in Buffer A: 20mM Hepes buffer, pH 7.5, 5mM Mg acetate, 30 mM β-mercaptoethanol, 100 µM phenylmethyl sulfonyl fluoride (PMSF), 10% glycerol and 0.5% Nonidet P-40 (NP40). Nuclei and unbroken cells were eliminated by centrifugation at 1,500γ for 10 min. The supernatant (S1.5) was centrifuged 10 min at 15,000γ in an Eppendorf Microfuge to obtain mitochondria and lysosomes-free supernatant (S15). Protein concentrations were measured by Micro-assays (Bradford, 1976).

Centrifuation of S15 for 2 hours at 100,000γ in a Beckman refrigerated ultracentrifuge was used to prepare cell sap (S100) and microsomes (P100) fractions.

EXAMPLE 15

ASSAY OF THE (2'-5') OLIGO A SYNTHETASE

Aliquots of S15 containing 1-2 µg protein were incubated in 20µl reactions containing 25mM Hepes buffer, pH 7.5, 20mM Mg acetate, 1mM dithiothreitol, 1.5mM ATP, 4µCi of ^{32}P-α-ATP, 50µg/ml poly(rI)(rC) (from PL-Biochemicals) for 2 hours at 30°C. After boiling for 5 min and Microfuge centrifugation, Bacterial Alkaline Phosphatase was added at 25 U/ml to an aliquot and the reaction incubated for 2 hours at 37°C. From 2 to 7µl were spotted on Whatmann 3MM paper and analyzed by electrophoresis at 3,000 V in pyridine/acetic acid pH 3.5. After autoradiography, the (A2'p)nA oligomers spots were cut out and counted.

EXAMPLE 16

ELECTROPHORETIC-TRANSFER IMMUNOBLOTS

Aliquots of crude cellular fractions (30µg protein) were adjusted

with Laemmli's sodium-dodecyl-sulphate-polyacrylamide gel electrophoresis loading buffer (Laemmli, 1970) and boiled 10 min before electrophoresis on 7.5 or 10 % gels. Ainersham's ^{14}C-methylated proteins were used as molecular weight standards (10^4 cpm). Electrophoretic transfer onto nitrocellulose paper (Schleicher and Schull BA85) was carried out in 25 mM Tris-base, 192mM glycine and 20% methanol. The blots were preincubated in 0.09M NaCl, 0.01M Tris-HCl pH 7.5, 10% (v/v) of a 1% fat milk solution, 10% (v/v) heat-inactivated fetal calf serum and 0.05% Tween-20, either for 2 hours at 37°C or overnight at 4°C followed by 30 min at 37°C. Blots were then incubated with anti-(2'-5') oligo A synthetase peptide B antibodies in form of rabbit IgG 0.1mg/ml, for 2 hours at 37°C. Blots were washed 5 times for 10 min in 4% fetal calf serum and incubated in the complete above preincubation mixture containing 10^6 cpm/ml of ^{125}I-protein A (Amersham, 30 mCi/mg) for 1 hour at 37°C Blots were washed and subjected to autoradiography.

EXAMPLE 17

IMMUNOPRECIPITATION OF (2'-5') OLIGO A SYNTHETASE ACTIVITY AND LABELED PROTEINS

First aliquots of 5-10µl anti-(2'-5') oligo A synthetase peptide B rabbit serum were adsorbed on 3mg of Protein A-Sepharose (Pharmacia) equilibrated in PBS with 3% Bovine serum albumin (BSA), for 30 min at room temperature, then washed with PBS-1% BSA. For immunoprecipitation of the (2'-5') oligo A synthetase enzymatic activity, aliquots of 2µg of S15 proteins in a final volume of 20µl of buffer A were adsorbed on the above pelleted IgG-Protein A Sepharose for 2 hours at 4°C. The suspension was diluted 5 fold in buffer A and the supernatant transferred to another tube. The pellet was washed 3 times with 0.5 ml buffer A and was then suspended in 25µl of the enzyme reaction mixture (see above). Activity was measured also on aliquots of the non-bound supernatant.

For labeling (2'-5') oligo A synthetase, Wish cells were grown to confluent monolayers on 3cm plastic dishes and treated for 12 hours with 500 U/ml rIFN-β1. The medium was replaced by 0.5 ml

methionine-free DMEM (GIBCO) containing 500µCi of ^{35}S-methionine (Amersham 400 mCi/mmol) and cells incubated for 2 hours, washed with PBS and homogenized in Buffer A. The S15 was used for immunoprecipitation. About 10^7 cpm of S15 proteins were added to the pelleted Portein A-Sepharose and mixed for 2 hours at 4°C. The Beads were washed with 1% BSA, 1% NP40, 2M KCl in PBS and twice with PBS only. Samples were analysed by sodium-dodecyl-sulphate-polyacrylamide gel electrophoresis.

EXAMPLE 18

USES OF ANTI-PEPTIDE ANTIBODIES TO DETECT THE (2'-5') OLIGO A SYNTHETASE ACTIVITY

Immunoprecipitation of the (2'-5') oligo A synthetase activity by anti-(2'-5') oligo A synthetase activity peptide antibodies

The antiserum B was raised against a peptide common to the E16 and E18 sequences, while antiserum C was raised against a peptide found only in E16. We used these antibodies to verify that they immunoprecipitate the (2'-5') oligo A synthetase activity specifically. The (2'-5') oligo A synthetase activity adsorbed on the immune IgG-Protein A Sepharose and that remaining in the supernatant were compared to the same fractions obtained by using non-immune IgG. Extracts from two cell lines which express preferentially either the 1.6 kb RNA (Wish cells) or the 1.8 kb RNA (Daudi cells) were compared. Antibodies C (E16-specific) were 20 times more efficient to adsorb the activity from Wish cells than normal serum. Substracting the background with normal serum, allows to evaluate what is specifically bound to anti-B and anti-C (Fig. 16). Anti-C retained the (2'-5') oligo A synthetase activity from Wish cells but not from Daudi cells, in line with the absence of E16 mRNA and 40 kd protein in these cells (see Example19). Anti-B adsorbed (2'-5') oligo A synthetase activity from both Daudi and Wish cell extracts.

The antibodies produced against peptides deduced from the cloned cDNAs, recognize, therefore, specifically different (2'-5') oligo A synthetase forms.

EXAMPLE 19

IMMUNOBLOT ANALYSIS OF THE DIFFERENT FORMS OF (2'-5') OLIGO A SYNTHETASE ACTIVITY FROM HUMAN CELLS

The antibodies against peptide B were tested for their ability to bind specifically to (2'-5') oligo A synthetase activity in crude extracts of human cells separated by sodium-dodecyl-sulphate-polyacrylamide gel electrophoresis and blotted electrophoretically onto nitrocellulose paper. In addition to being recognized by the antibodies in immunoblots, we expect genuine (2'-5') oligo A synthetase proteins present in extracts of these human cells to be induced by IFN treatment, and we therefore looked only at the induced proteins revealed by the immunoblots (Fig. 17).

The cell lines Daudi, Wish and SV80 were compared because of their differences in the pattern of expression of the (2'-5') oligo A synthetase mRNAs (see Example 18). Antibodies B detect as expected a 45-46 kd protein similar in size to the E18 product, in Daudi cells and no 40 kd which would correspond to E16 whose mRNA is not expressed by Daudi cells. In contrast, the 40 kd E16 protein is present in Wish cells without 46 kd E18 in line with the absence of 1.8 kb RNA in these cells. Both proteins are detected by anti-B in SV80 cells. These results demonstrate that human cells produce the 40 and 46 kd proteins and this only when they express the 1.6 and 1.8 kb RNAs respectively.

The immunoblots with anti-B also reveal that there are not only two forms of (2'-5') oligo A synthetase in human cells but probably four different forms. This can be deduced from the fact that in addition to the 40 and 46 kd proteins, anti-B clearly detected two other proteins of 100 kd and 67 kd which are induced by IFN (Fig. 17 and 18). The 100 kd was not detected in Daudi cells, showing that the large proteins detected by anti-B are also expressed in a cell-specific pattern. The fact that the anti-B was raised against a peptide derived from the sequence of genuine (2'-5') oligo A synthetase forms, and that the two larger proteins are induced by IFN, makes it very likely that they belong to the (2'-5') oligo A

synthetase system. To ascertain that these are (2'-5') oligo A synthetase forms, we have purified (2'-5') oligo A synthetase from different cellular fractions of SV80 and followed in parallel the protein detected by antibodies B.

Separation of the different active forms of (2'-5') oligo A synthetase

The separation of the different protein species detected by antibodies B is shown in Fig. 18 and 19. Most of the 40 kd protein remains in the 100,000g supernatant (S100) of NP40 cytoplasmic extraxts from SV80 cells. It is not adsorbed on DEAE-cellulose in low salt and adsorbs to CM-cellulose from which it elutes at high salt concentration. In contrast, the 100 kd protein is almost absent from S100 and is concentrated in the microsomal pellet (P100) from which it can be solubilized by Na deoxycholate (DOC) or 0.5M KCl. This protein was adsorbed on DEAE-cellulose at low salt and elutes at high salt. The 67 kd and 45-46 kd remain partly in S100 but are relatively concentrated per mg protein in the microsomal pellet. They appear to be less readily extractable from microsomes by DOC or KCl.

Sedimentation on glycerol gradients showed that the activity purified from S100 after CM-cellulose, parallels the sedimentation of the 40 kd protein E16. The fraction purified from P100 and eluted from DEAE-cellulose, containing the 100 and 46 kd proteins, separated into two peaks on glycerol gradients, sedimenting as 80,000 and 45,000 Mr proteins. The (2'-5') oligo A synthetase in the heavy peak parellels the presence of the 100 kd protein.

Different enzymatic properties of the various (2'-5') oligo A synthetase activity forms

The 40 kd protein from the glycerol gradient has an optimal pH of 6.8 for its activity, and is only 25% as active at pH 7.8. Moreover, no activity of the 40 kd (2'-5') oligo A synthetase can be observed at concentrations of poly (rI)(rC) lower than 1µg/ml and the maximal activity requires 50-100µg/ml (Fig.20). The same high ds RNA requirement was found for the E16 cDNA product produced by recom-

binant DNA technology in E.coli.

The 100 kd protein after the glycerol gradient, has an optimal pH of 7.6 for its (2'-5') oligo A synthetase and is less active at acidic pH. It is maximally active already at extremely low concentrations of poly (rI)(rC) or in its absence, and its activity is even inhibited by high ds RNA concentrations. This strongly suggests that the different (2'-5') oligo A synthetase forms, because of their different cytoplasmic localizations and enzymatic properties, are used by the cells under different conditions.

Many observations suggest that the IFN-induced (2'-5') oligo A synthetase is involved in two distinct, seemingly opposite, phases of cell growth (cell.cycling and growth inhibition) in addition to its possible role in the antiviral effect (reviewed in Revel, 1984). This may be relevant to the issue of multiple (2'-5') oligo A synthetase forms. In synchronized cell cultures we have observed that (2'-5') oligo A synthetase behaves as a cell-cycle protein (Mallucci et al, 1985). Thus, synchronized cultures of Mouse embryo fibroblasts exhibit a sharp rise in (2'-5') oligo A synthetase activity and (2'-5') oligo A synthetase mRNA at the end of the S-phase followed by a rapid disappearance of the RNA and enzyme activity when the cells proceed to G2. Anti-mouse IFN antibodies reduced the (2'-5') oligo A synthetase induction. In this system we also observed that the (2'-5') oligo A synthetase RNA which accumulates in S-phase is a large 4-5 kb transcript different from the 1.7 kb RNA species which accumulates in the same cells when treated with exogenous IFN. This suggests that the S-phase (2'-5') oligo A synthetase is a different form of the enzyme than that in cells growth-arrested by exogenously added IFN. Because of its large mRNA, it is likely to be like the 100 kd, a low ds RNA requiring form. Anti-B antibodies detected also the (2'-5') oligo A synthetase multiple forms in mouse cells.

These considerations illustrate the advantage of being able to assay independently the 4 forms of (2'-5') oligo A synthetase, which may vary individually in various physiological conditions and

diseases.

EXAMPLE 20

USE OF ANTI-(2'-5') OLIGO A SYNTHETASE ACTIVITY PEPTIDES ANTIBODIES FOR IMMUNOASSAYS OF (2'-5') OLIGO A SYNTHETASE ACTIVITY

Since the anti-B antibodies recognizes all the forms of (2'-5') oligo A synthetase, it can be used for an immunoassy of (2'-5') oligo A synthetase in unfractionated extracts of human cells either from cultures or directly obtained from patients.

An example of a solid-phase radio-immunoassay is shown in Fig. 21. Wish and Daudi cells, either treated by IFN or untreated, were lysed by the NP40-containing Buffer A and S15 prepared by microfuge centrifugation as described above. Aliquots containing 1 to 10µg of protein were directly applied to nitrocellulose paper (or to other protein-binding paper) and the sheet treated with anti-B as for regular immunoblots (Example 19). The autoradiography in Fig. 11 shows that ^{125}I-Protein A binds only to the samples originating from IFN-treated cells. It is clear that this assay could be used also in form of enzyme-linked immunoassay (ELISA) by replacing the labeled Protein-A by Peroxydase or β-galactosidase conjugated anti-rabbit IgG.

The immunoassay of (2'-5') oligo A synthetase is rapid: 20 min for cell extract preparation, 2 hours for anti-B and Protein-A adsorption and washing. The assay is sensitive and very small amounts of cell extracts suffice to measure that (2'-5') oligo A synthetase level. It is specific, no signal being obtained in non-IFN treated cells.

EXAMPLE 21

IMMUNOFLUORESCENCE MICROSCOPY DETECTION OF (2'-5') OLIGO A SYNTHETASE IN CELLS

Enzymatic assays have established (see prior art) that (2'-5') oligo A synthetase is elevated in peripheral blood monomuclear

cells of patients with viral infections. The anti-(2'-5') oligo A synthetase peptide antibodies can be used in immunofluorescence microscopy to detect (2'-5') oligo A synthetase elevation in cells in general, and white blood cells in particular.

Blood (2 ml) was withdrawn from a healthy donor and from a patient with acute viral illness. The mononuclear blood cells were separated by Ficoll-Hypaque (Pharmacia) centrifugation, and spread on glass coverslips directly or by the use of a cytospin microfuge (microhematocrite). The cells were washed in PBS, fixed for 30 min in 3% paraformaldehyde at room temperature (RT), rinsed with PBS and treated with 0.5% Triton-X100 in Hank's salts for 5 min, rinsed again with PBS and with PBS-2% gelatin. The coverslips were then incubated with antiB serum diluted 1:5 in PBS-gelatin applied as a 40µl droplet on parafilm onto which the coverslip were deposited. After 60 min RT, the coverslips were rinsed in PBS-gelatin and FITC conjugated anti-rabbit IgG (BioYeda) diluted 1:20 was applied by the parafilm procedure. After 20 min at RT, coverslips were washed twice with PBS-gelatin, then with $H_2O$and mounted on microscopic slides with Miviol 4-88 (Hoechst)-Glycerol (2.5g Moviol, 6g Glycerol and 6 ml H_2O to which 12 ml of 0.2M Tris.HCl pH 8.5 were added, followed by incubation at 50°C and clarification 15 min at 5,000γ). Parallel coverslips were processed using normal rabbit serum instead of anti-B. Slides were observed in a Zeiss fluorescence microscope and photographed on Polaroid film with 30 seconds exposures.

Fig. 22 shows that the lymphocytes were stained with anti-(2'-5') oligo A synthetase in blood samples from the patient with viral infection but not in the blood of the healthy donor, where only a low fluorescence of macrophages and granulocytes is seen. Normal serum did not stain lymphocytes but also gave a low background in macrophages/granulocytes. Thus, the present anti-(2'-5') oligo A synthetase peptide antibodies can be used for microscopic observation of cells, in cultures, blood and tissue sections, to evaluate if the cells have reacted with interferon and have accumulated (2'-5') oligo A synthetase.

REFERENCES

- Baglioni C. and Nilsen T.W. (1983) in Gresser, I. (ed) Interferon 5, Acad. Press, New York, pp 23 - 42.

- Ball L.A. (1980) Ann.N.Y.Acad.Sci. 350, 486 - 496.

- Barany G, Merrifield R (1980). Solid phase Peptide Synthesis. In Gross E, Meienhofer J (eds): "The Peptides: Analysis, Synthesis and Biology". New York: Acad press. 2:1-284.

- Benech, P., Merlin, G., and Chebath, J. (1985) Nucl. Acids Res. 13, 1267-1281.

- Bradford M (1976). Anal Biochem 72:248.

- Breathnach, R. and Chambon, P. (1981) Ann.Rev.Biochem. 50, 349-383.

- Chebath, J., Merlin, G., Metz, R., Benech, P. and Revel M. (1983) Nucl.Acids Res. 11, 1213-1226.

- Cheley, S., and Anderson, R. (1984) A reproducible microanalytical method for the detection of specific RNA sequences by dot-blot hybridization. Anal Biochem. 137, 15-19.

- Chernajovsky Y, Mory Y, Chen L, Marks Z, Novick D, Rubinstein M, Revel M (1984). Efficient Constitutive Production of Human Fibrolast Interferon by Hamster Cells Transformed with the IFN-β1 Gene Fused to an SV40 Early Promoter. DNA 3:297-308.

- Creasey A., Eppstein D.A., Marsh Y.V., Khan Z. and Merigan T.C. (1983) Mol.Cell.Biol. 3, 780-786.

- Degrave W., Derynck R., Tavernier J., Haegeman G. and Fiers W. (1981) Gene 14, 137-143.

- Dougherty J., Samanta H., Farrell P. and Lengyel P. (1980) J.Biol.Chem. 255, 3813-3816.

- Epstein D.A., Czarniecki W., Jacobson H., Friedman R.M. and Panet A. (1981) Eur.J.Biochem. 118, 9-15.

- Etienne-Smekens M., Goldstein J., Ooms H. and Dumomt J. (1983) Eur.J. Biochem. 130, 269-273.

- Fellous M., Nir U., Wallach D., Merlin C., Rubinstein M. and Revel M. (1982) Proc.Natl.Acad.Sci.USA 79, 3082-3086.

- Ferbus D., Justesen J., Besancon F. and Thang M.N. (1981) Biochem.Biophys.Res.Commun. 100, 847-856.

- Friedman R.L., Manly S.P., McMahon M., Kerr I.M. and Stark G.R. (1984) Cell 38, 745-755.

- Green M., Maniatis T. and Melton D.A. (1983) Cell 32, 681-694.

- Green N., Alexander H., Olson A., Alexander S., Schinnick T., Sutcliffe G., Lerner R. (1982). Cell 28: 477-487.

- Hovanessian A.G., Brown R.E. and Kerr I.M. (1977) Nature 268, 537-540.

Johnston M., Friedman R.M. and Torrence P.F. (1980) Biochemistry 19, 5580-5585.

- Karin M. and Richards R.I., (1982) Nature 299, 797-802.

- Keller W. (1984) Cell 39, 423-425.

- Kerr I.M. and Brown, R.E. (1978) Proc. Natl.Acad.Sci. USA 75, 256-260.

- Kimichi A., Shure H., Lapidot Y., Rapoport S., Panet A and Revel M. (1981) FEBS Lett. 134, 212-216.

- Kitamura N., Semler B.L., Rothberg P.G., Larsen G.R., Adler C.J., Dorner A.J., Emini E.A., Hanecak R., Lee J.J., van der Werf S., Anderson C.W. and Wimmer E. (1981), Nature 291:547-553.

- Kozak M. (1984) Nature 308, 241-246.

- Kyte J. and Doolittle R.F. (1982) J.Mol.Biol. 157, 105-132.

- Laemmli W.K. (1970), Nature 227:680-685.

- Laurence L., Marti J., Roux D. and Cailla H. (1984) Proc.Natl.Acad.Sci.USA 81, 2322-2326.

- Lebleu B. and Content J. (1982) in Gresser, I. (ed) Interferon 4, Acad. Press, New York, pp 47-84.

- Lengyel P. (1982), Ann. Rev. Biochem. 51: 251-282.

- Malissen M., Malissen B. and Jordan B. (1982), Proc. Natl. Acad. Sci. USA 79, 893-897.

- Mallucci L., Chebath J., Revel M., Wells V., Benech P. (1985). Interferon and 2-5A Synthetase Gene Expression During The Cell Cycle. In: Dianzani F, Roosi GB (eds): "The Interferon System", Serono Symposia Vol 24. New York: Raven Press, pp 165-170.

- Maniatis T., Hardison R.,, Lacy E., Lauer J., O'Connell C., Quon D., Gek Kee S. and Efstradiatis A. (1978) Cell 15, 687-701.

- Maniatis T., Fritsch P. and Sambrook J. (1982) Molecular Cloning: A laboratory manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y..

- Maxam A.M. and Gilbert W. (1980) Meth.Enzymol. 65, 499-560.

- Merlin G., Chebath J., Benech P., Metz P. and Revel M. (1983) Proc.,Natl.Acad.Sci.USA 80, 4904-4908.

- Merritt JA, Borden EC, Ball LA (1985). Measurements of 2'-5'Oligoadenylate Synthetase in Patients Receiving Interferon-Alpha. J Interferon Res 5:191-198.

- Minks M.A., West D.K., Benvin S. and Baglioni C. (1979) J.Biol.Chem. 254, 10180-10183.

- Mory Y., Chernajovsky Y., Feinstein S.L., Chen L., Nir., U., Weissenbach J., Malpiece Y., Tiollais P., Marks D., Ladner M., Colby C. and Revel M. (1981) Eur. J.Biochem. 120, 192-202.

- Nilsen T.M., Maroney P.A., Robertson H.D. And Baglioni C. (1982a) MolCell.Biol. 2, 154-160.

- Nilsen T.M., Wood D.L. and Baglioni C. (1982b) J.Biol. Chem. 257, 1602-1605.

- Nir U., Cohen B., Chen L. and Revel M. (1984) Nucl. Acids Res. 12, 6979-6993.

- Pustell J. and Kafatos F.C. (1982a) Nucl. Acids Res. 10, 4765-4782.

- Pustell J. and Kafatos F.C. (1982b) Nucl. Acids Res. 10, 51-59.

- Raj N.B.K. and Pitha P.N. (1981), Proc. Natl. Acad.

Sci. USA 78: 7426-7430.

- Read SE, Williams BRG, Coates RA, Evans WK, Fanning MM, Garvey MB, Shepherd FA (1985). Elevated levels of Interferon-induced 2'-5'Oligoadenylate Synthetase in Generalized Persistent Lymphoadenopathy and the Acquired Immunodeficiency Syndrome. J Infect Dis 3:466-472.

- Revel M. (1984) in Becker, Y. (ed) Antiviral Drugs and Interferons: The Molecular basis of their activity, Martinus Nijhoff, Boston, pp 357-433.

- Revel M., Kimchi A., Shulman L., Fradin A., Shuster R., Yakobson E., Chernajovsky Y., Schmidt A., Shure H. and Bendori R. (1980) Ann.N.Y.Acad.Sci. 350, 459-472.

- Revel M., Wallach D., Merlin G., Schattner A., Schmidt A., Wold D., Shulman L. and Kimchi A. (1981) Meth. Enzymol. 79, 149-161.

--Revel M., Kimchi A., Friedman M., Wold D., Merlin G., Panet A., Rapoport S. and Lapidot Y. (1982) Texas Rep. Biol.Med. 41, 452-462.

- Revel M., Shattner A., Wallach D., Merlin G., Levavi H., Hahn T., Levin S., (1982). Monitoring of Interferon Therapy, Diagnosis of viral diseases and detection of Interferon Deficiencies by Assay of an Interferon-induced enzyme in Human peripheral White Blood Cells. In Kono R, Vilcek J (eds): "The Clinical Potential of Interferons". Tokyo: University of Tokyo Press, pp 353-367.

- Rigby P., Dieckman M., Rhodes C. and Berg P. (1977) J.Mol.Biol. 113, 237-251.

- Rosa F.,Berissi H., Weissenbach J., Maroteaux L.,

Fellous M. and Revel M. (1983a) EMBO J. 2, 239-243.

- Rosa F., Hatat D., Abadie A., Wallach D., Revel M. and Fellous M. (1983b) EMBO J. 2, 1585-1589.

- Salzberg S., Wreschner D.H., Oberman F., Panet A. and Bakhanaschvili M. (1983) MolCell.Biol. 3, 1759-1765.

- Samanta H., Dougherty J.P. and Lengyel P. (1980) J. Biol.Chem.255, 9807-9813.

- Samanta H., Pravtcheva D.D., Ruddle F.H. and Lengyel P. (1984) J. Interferon Res. 4, 295-300.

- Saunders M.E. and Williams B.R.G. (1984) Antiviral Res. ABSTR No 3, p. 60.

- Schamboeck A., Korman A.J., Kamb A. and Strominger J. L. (1983) Nucl. Acids Res. 11, 8663-8675.

- Schattner A., Merlin G., Wallach D., Rosenberg H., Bino T., Hahn T., Levin S., Revel M. (1981a) Monitoring of Interferon therapy by assay of 2'5' oligoisoadenylate synthetase in human peripheral white blood cells, J. Interferon Res. 1, 587-594.

- Schattner, A., Wallach D., Merlin G., Hahn T., Levin S., Revel M. (1981b) Assay of an interferon-induced enzyme in white blood cells as a diagnostic aid in viral diseases. Lancet 2, 497-500.

- Schattner A., Merlin G., Shapira A., Revel M., Wallach D. (1982a) Comparison of (2'-5') Oligoadenylate Syntheses and Interferon Blood-Levels in Mice early after viral Infection. J Interferon Res 2:285-289.

- Schattner A., Wallach D., Merlin G., Hahn T., Levin S., Ramot B., Revel M. (1982b). Variations of (2'-5')

oligo A synthetase level in lymphocytes and granulocytes of patients with viral infections and leukemia. J. Interferon Res. 2, 355-361.

- Schattner A., Merlin G., Bregman U., Hahn T., Levin S., Revel M., Wallach D. (1984). (2'-5') oligo A synthetase in human polymorphonuclear cells increased activity in interferon treatment and in viral infections. Clin.Exp.Immunol. 57, 265-270.

- Schoenfeld A., Nitke S., Schattner A., Wallach D., Crespi M., Hahn T., Levavi H., Ovadia J., Shoham J., Doerner T., Revel M. (1984). Intramuscular human interferon-β_1 injections in treatment of condylomata acuminata, Lancet i, 1038-1042.

- Schoenfeld A., Ovadia J., Levavi C., Nitke S., Wallach D., Schattner A., Revel M. (1985). Indentification of viral infections in pregnancy by assay of (2'-5') oligoadenylate synthetase. Early Human Develop. 10, 279-286.

- Schmidt A., Zilberstein A., Shulman L., Federman P., Berissi H. and Revel M. (1978) FEBS Lett. 25, 257-264.

- Shulman L.M., Barker P.E., Hart J.T., Messer Peter P.G. and Ruddle F.H. (1984). Somatic Cell Mol.Genet. 10, 247-257.

- Shulman L. and Revel M. (1980), Nature 287:98-100.

- Stark G., Dower W.J., Schimke R.T., Brown R.E. and Kerr I.M. (1979) Nature 278: 471-473.

- Spirer Z., Zakut R., Bogair N., Fridkin M. (1977). Eur J Immunol 7:69-74.

- StLaurent G., Yoshie O., Floyd-Smith G., Samanta H.,

Sehgal P. and Lengyel P. (1983) Cell 33, 95-102.

- Strachan T., Sodoyer R., Damotte M. and Jordan B.R. (1984), EMBO J. 3: 877-894.

- Thomas (1980), proc. Natl. Acad. Sci. USA 77: 5201-5205.

- Trifonov, E.N. (1984) Codata Bulletin 56, 21-26.

- Wallach D. and Revel M. (1980) Nature 287, 68-70.

- Wallach D., Fellous M. and Revel M. (1982), Nature 299: 833-836.

- Wallach D., Schattner A., Merlin G., Kimchi A., Fellous M. and Revel M. (1982) in Merigan, T.C., Friedman, R.M. (eds) Interferons, UCLA Symposia Vol. XXV, Acad. Press, New York, pp 449-463.

- Wallach D., Aderka D., Budilovsky S. and Hahn T. (1983) in DeMaeyer, E., Schellekens, H. (eds) The Biology of the Interferon System, Elsevier Sci.Publ., Amsterdam, pp 293-303.

- Weil J., Epstein C.J., Epstein L.B., Sednak J.J., Sabran J.L., Grossberg S.E. (1983) Nature 301, 437-439.

- Weissenbach J., Zeevi M., Landau T. and Revel M. (1979), Eur. J. Biochem 98: 1-8.

- Wells V. and Mallucci L. (1985) J.Cell.Biol., in press.

- Wigler M., Sweet R., Sim G.K., Wold B., Pellicer A., Lacy E., Maniatis T., Silverstein S. and Axel R. (1979) Cell 16, 777-785.

- Williams BRG and Read SE (1981). Detection of elevated levels of the interferon-induced enzyme, 2-5 synthetase,

in infectious disease and in purturition. In DeMaeyer E., Galasso G., Schellekens H. (eds): "The Biology of the Interferon System", Elsevier/North Holland Biomedical Press, pp 111-114.

- Wolf D. and Rotter V. (1985) Proc.Natl.Acad. Sci.USA 82, 790-794.

- Yang K., Samanta H., Dougherty J., Yayaram B., Broeze R. and Lengyel P. (1981) J.Biol.Chem. 256, 9324-9328.

- Yarden A., Shure-Gottlieb H., Chebath J., Revel M. and Kimchi A. (1984) EMBO J. 3, 969-973.

- Zilberstein A., Kimchi, A. Schmidt A. and Revel M. (1978) Proc.Natl.Acad.Sci.USA 75, 4734-4738.

- Zinn K., DiMaio D. and Maniatis T. (1983) Cell 34, 865-879.

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CLAIMS

1. A human DNA sequence coding for an enzyme having (2'-5') oligo A synthetase activity.

2. A human DNA sequence according to claim 1, coding for an enzyme having the amino acid sequence set forth in Fig. 7A.

3. A human DNA sequence according to claim 1, coding for an enzyme having the amino acid sequence set forth in Fig. 7B.

4. A human DNA sequence according to claim 1, the enzyme consisting of a C-terminal heptadecapeptide common to the amino acid sequences mentioned in claims 2 and 3.

5. A human DNA sequence according to claim 1, showing the restriction map set forth in Fig. 9.

6. A sequence of (2'-5') oligo A synthetase cDNA set forth in Fig. 7A being complementary to an 1.6 kb mRNA.

7. A sequence of (2'-5') oligo A synthetase cDNA set forth in Fig. 7B being complementary to an 1.8 kb mRNA.

8. A DNA sequence which comprises the promoter of the DNA sequence according to one of the claims 1 to 5.

9. A DNA sequence according to claim 8, having the restriction map set forth in Fig. 10.

10. A DNA sequence according to claims 8 or 9 having the DNA sequence set forth in Fig. 11.

11. A 1.6 kb RNA having a nucleotide sequence complementary to the nucleotide sequence set forth in Figure 7A.

12. A 1.8 kb RNA comprising a nucleotide sequence complementary to the sequence of nucleotides 1-1322 set forth in Figure 7A and the sequence of nucleotides 901-1590 set forth in Figure 7B.

13. A DNA transfer vector comprising an inserted DNA sequence consisting essentially of the cDNA sequence of claim 6.

14. A DNA transfer vector according to claim 13, which is λgt 11-E16 in which the inserted cDNA sequence has been fused in phase with the lac Z gene suitable for expression of the coding sequence.

15. A DNA transfer vector comprising an inserted DNA sequence consisting essentially of the cDNA sequence of claim 7.

16. A micororganism transformed by an expression vector according to one of the claims 13 to 15.

17. A microorganism according to claim 16, which is Escherichia coli.

18. An enzyme having (2'-5') oligo A synthetase activity and the amino acid sequence set forth in Figure 7A.

19. An enzyme having (2'-5') oligo A synthetase activity which comprises the sequence of amino acids 1-364 set forth in Figure 7A and the sequence of amino acids 290-400 set forth in Figure 7B.

20. The enzyme of claim 18 comprising about 364 amino acids and having a molecular weight of about 41,500 daltons.

21. The enzyme of claim 19 comprising about 400 amino acids and having a molecular weight of about 46,000 daltons.

22. A method of monitoring the response of a patient to an interferon, which comprises measuring the concentration of (2'-5') oligo A synthetase mRNA in cells or body fluids of the patient by hybridizing to the mRNA DNA complementary thereto.

23. The method of claim 22, wherein the mRNA is the 1.6 kb RNA of claim 11.

24. The method of claim 22, wherein the mRNA is the 1.8 kb RNA of claim 12.

25. A method for evaluating the response of cells and tissues to interferon which comprises hybridizing RNA from cells or tissues exposed to interferon with cDNA complementary to the RNA, and determining the extent of hybridization.

26. A method according to claim 25, wherein the RNA is extracted from cells or tissues which have been exposed to interferon, immobilized on a membrane filter and hybridized to labelled cDNA specific for interferon-induced mRNAs.

27. A method according to claim 25, which comprises <u>in situ</u> hybridization of labelled cDNA to slices of tissues and evaluating by microscopic examination autoradiography, or fluorescence.

28. A method according to claim 25, wherein the cells or tissues are of human origin.

29. A kit for carrying out a method according to claim 25, containing a cDNA complementary to a sequence set forth in Figure 7A or 7B, reagents to carry out the hybridization tests for nick-translation with deoxyribonuclease I and [^{32}P]-gamma-dCTP, reagents for hybridization on nitrocellulose membranes, and reagents for RNA extraction from cells.

30. An antigenic peptide having an amino acid sequence contained within the amino acid sequence set forth in Figure 7A.

31. An antigenic peptide having an amino acid sequence contained within the sequence of amino acids 1-364 set forth in Figure 7A and the sequence of amino acids 290-400 set forth in Figure 7B.

32. The antigenic peptide of claim 30, having the amino acid sequence comprising the 17 C-terminal amino acids of the amino acid sequence set forth in Figure 7A and having the amino acid sequence: ARG-PRO-PRO-ALA-SER-SER-LEU-PRO-PHE-ILE-PRO-ALA-PRO-LEU-HIS-GLU-ALA.

33. An antigenic peptide of claim 30, having the amino acid sequence:
GLU-LYS-TYR-LEU-ARG-ARG-GLN-LEU-THR-LYS-PRO-ARG-PRO-VAL-ILE-LEU-ASP-PRO-ALA-ASP.

34. An antibody raised against the antigenic peptide of claim 30, which recognizes and immunoprecipitates (2'-5') oligo A synthetase.

35. An antibody raised against the antigenic peptide of claim 31 which recognizes and immunoprecipitates (2'-5') oligo A synthetase.

36. An antibody according to one of the claims 34 or 35 against all four of the 40 KD, 46 KD, 67 KD and 100 KD forms of (2'-5') oligo A synthetase.

37. An antibody of claim 36, said antibody being obtained by immunizing an animal with peptide B.

38. An antibody of claim 36, said antibody being conjuated with a label to form a labeled antibody.

39. An antibody of claim 38, wherein the label is a fluorescent label.

40. An antibody of claim 38, wherein the label is a radioactive label.

41. An antibody of claim 38, wherein the label is an enzyme.

42. An assay for the 40 KD, 46 KD, 67 KD and 100 KD forms of (2'-5') oligo A synthetase in cells which comprise incubating the cells with the labeled antibody of claim 38 and detecting cells bearing (2'-5') oligo A synthetase activity in any of said forms by means of said label.

43. An assay of claim 42 in which the assay is a fluorescent immunoassay.

44. An assay of claim 42 in which the assay is a radioimmunoassay

45. An assay of claim 42 in which the assay in an enzyme immunoassay.

46. An assay of claim 42 in which the cells are mononuclear blood cells.

47. A kit for the detection of all four forms of (2'-5') oligo A synthetase in cells comprising the antibody of claim 38.

48. A 67 KD (2'-5') oligo A synthetase protein in a state of enhanced purity.

49. A 100 KD (2'-5') oligo A synthetase protein in a state of enhanced purity.

50. An antibody against one of the 40 KD, 46 KD, 67 KD or 100 KD forms of (2'-5') oligo A synthetase which does not cross-react with the other three forms.

51. A method of monitoring interferon activity in a subject which comprises measuring the amount of (2'-5') oligo A synthetase in a cell or body fluid of the subject at predetermined time intervals, determining the differences in the amount of said synthetase in the cell or body fluid of the subject within the different time intervals, and determining therefrom the amount of synthetase in the cell or body fluid of the subject and thereby the interferon activity of the subject.

52. The method of claim 51, wherein the amount of synthetase is measured by contacting the synthetase with the antibody of claim 34 so as to form a complex therewith and determining the amount of complex so formed.

53. The method of claim 52 further comprising the extraction of (2'-5') oligo A synthetase from a cell or body fluid which has been exposed to interfereon, labelling the extracted synthetase with an identifiable marker to form a labelled synthetase, contacting the labelled synthetase with the antibody under suitable conditions so as to form a labelled-synthetase-antibody complex, and detecting the marker in the complex, thereby detecting the synthetase.

54. The method of claim 53 wherein the marker is ^{35}S-methionine.

55. A kit for carrying out the method of claim 53, comprising the antibody of claim 34 or 35, materials for extracting the synthetase, materials for labelling the synthetase, and materials for detecting the marker and determining the amount of synthetase.

56. Cloned DNA that specifically hybridizes to messenger RNAs which appear in human cells after exposure to interferon.

57. Cloned cDNA according to claim 56 specific for the (2'-5') oligo A synthetase mRNAs of 3.6, 1.8 and 1.6 kilobase.

58. Cloned DNA according to claim 56 specific for the mRNA of a 56,000 Mr-protein, which mRNA is 2 kilobase and which has the nucleotide sequence set forth in Figure 1.

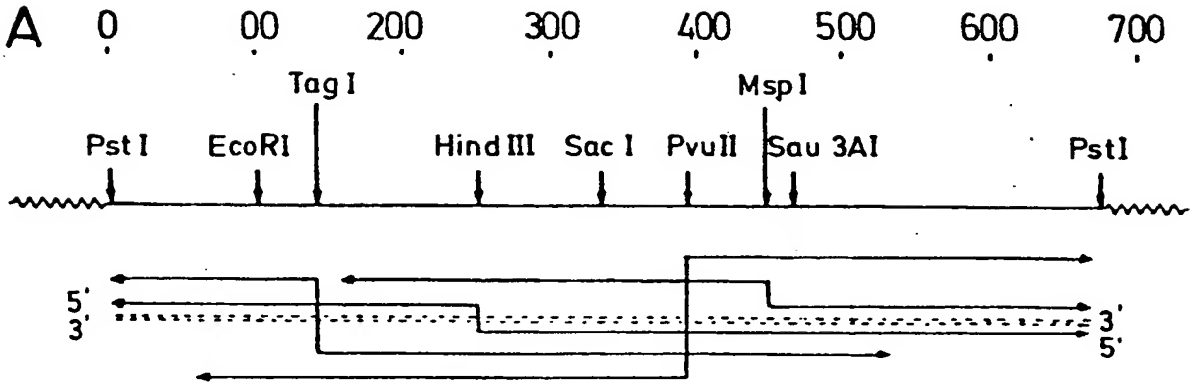

A
0
00
200
300
400
500
600
700
Tag I
Msp I
Pst I
EcoRI
Hind III
Sac I
PvuII
Sau 3AI
PstI
5'
3'
3'
5'

FIG. 1A

B

```
                                                                  30                                                                  60
TTT CGG ACG GTC TTG GAA TTA GTC ATA AAC TAC CAG CAA CTC TGC ATC TAC TGG ACA AAG
PHE ARG THR VAL LEU GLU LEU VAL ILE ASN TYR GLN GLN LEU CYS ILE TYR TRP THR LYS
                                                                  90                                                                 120
TAT TAT GAC TTT AAA AAC CCC ATT ATT GAA AAG TAC CTG AGA AGG CAG CTC ACG AAA CCC
TYR TYR ASP PHE LYS ASN PRO ILE ILE GLU LYS TYR LEU ARG ARG GLN LEU THR LYS PRO
                                                                 150                                                                 180
AGG CCT GTG ATC CTG GAC CCG GCG GAC CCT ACA GGA AAC TTG GGT GGT GGA GAC CCA AAG
ARG PRO VAL ILE LEU ASP PRO ALA ASP PRO THR GLY ASN LEU GLY GLY GLY ASP PRO LYS
                                                                 210                                                                 240
GGT TGG AGG CAG CTG GCA CAA GAG GCT GAG GCC TGG CTG AAT TAC CCA TGC TTT AAG AAT
GLY TRP ARG GLN LEU ALA GLN GLU ALA GLU ALA TRP LEU ASN TYR PRO CYS PHE LYS ASN
                                                                 270                                                                 300
TGG GAT GGG TCC CCA GTG AGC TCC TGG ATT CTG CTG GTG AGA CCT CCT GCT TCC TCC CTG
TRP ASP GLY SER PRO VAL SER SER TRP ILE LEU LEU VAL ARG PRO PRO ALA SER SER LEU
                                                                 330                                                                 360
CCA TTC ATC CCT GCC CCT CTC CAT GAA GCT TGA GAC ATA TAG CTG GAG ACC ATT CTT TCC
PRO PHE ILE PRO ALA PRO LEU HIS GLU ALA
                                                                 390                                                                 420
AAA GAA CTT ACC TCT TGC CAA AGG CCA TTT ATA TTC ATA TAG TGA CAG GCT GTG CTC CAT
                                                                 450                                                                 480
ATT TTA CAG TCA TTT TGG TCA CAA TCG AGG GTT TCT GGA ATT TTC ACA TCC CTT GTC CAG
                                                                 510
AAT TCA TTC CCC TAA GAG TAA TAA TAA ATA ATC TCT AAC ACC AAA
```

FIG.1B

0217102

0217102

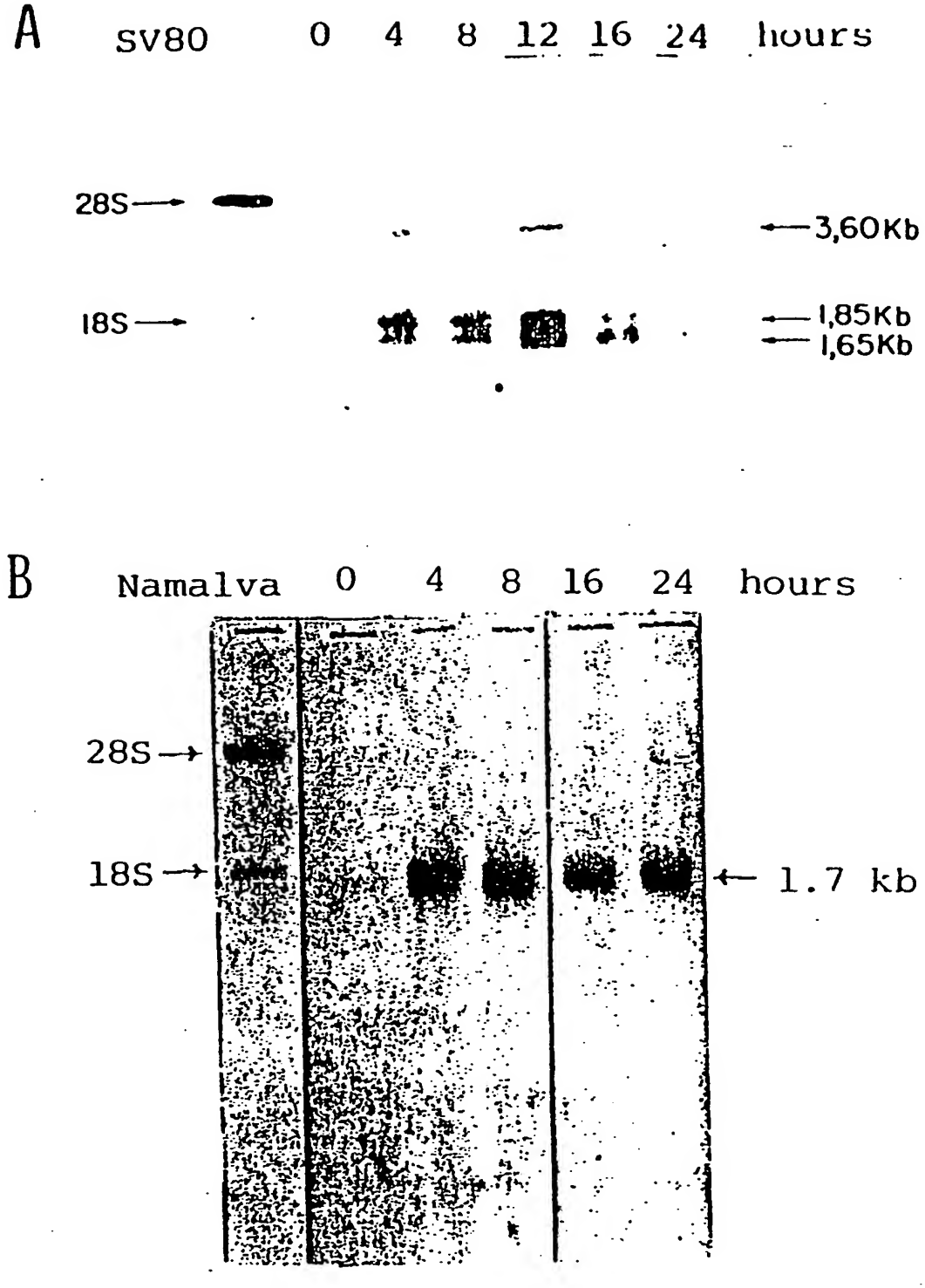


FIG.2

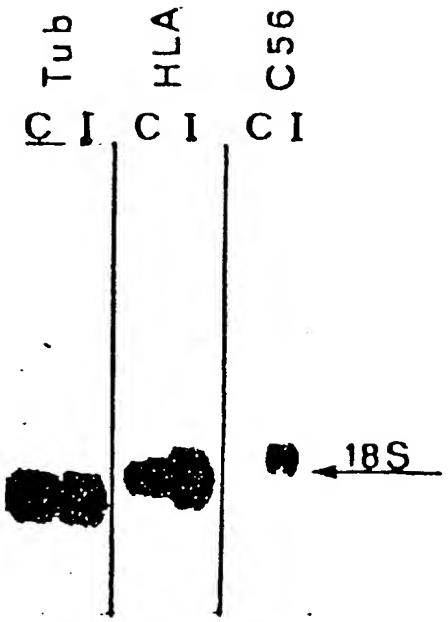


FIG.3

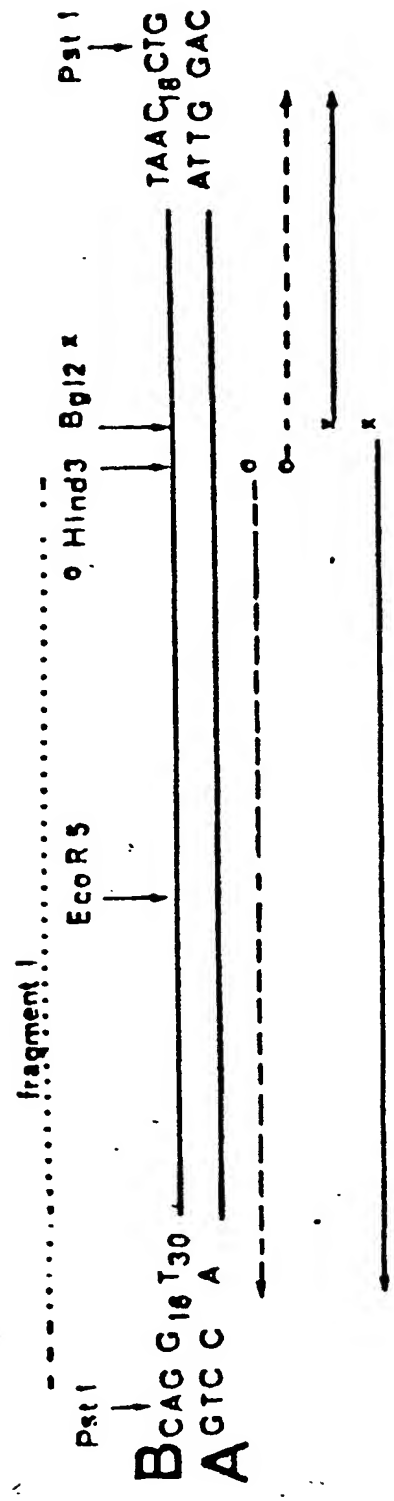

Pst I
TAAC18CTG
ATTG GAC
Bgl2
Hind3
EcoR5
fragment I
Pst I
B CAG G18 T30
A GTC C A

FIG. 4A

B

```
                                                30                                                  60
TTA ACA AGG GAT AAA AGT ATC AAT TCT TTG AAG AAA TTG GTT TTA AGG AAA CTT CGG AGA
    THR ARG ASP LYS SER ILE ASN SER LEU LYS LYS LEU VAL LEU ARG LYS LEU ARG ARG

                                                90                                                 120
AAG GCA TTA GAT CTG GAA AGC TTG AGC CTC CTT GGG TTC GTC TAC AAA TTG GAA GGA AAT
LYS ALA LEU ASP LEU GLU SER LEU SER LEU LEU GLY PHE VAL TYR LYS LEU GLU GLY ASN

                                               150                                                 180
ATG AAT GAA GCC CTG GAG TAC TAT GAG CGG GCC CTG AGA CTG GCT GCT GAC TTT GAG AAC
MET ASN GLU ALA LEU GLU TYR TYR GLU ARG ALA LEU ARG LEU ALA ALA ASP PHE GLU ASN

                                               210                                                 240
TCT GTG AGA CAA GGT CCT TAG GCA CCC AGA TAT CAG CCA CTT TCA CAT TTC ATT TCA TTT
SER VAL ARG GLN GLY PRO END

                                               270                                                 300
TAT GCT AAC ATT TAC TAA TCA TCT TTT CTG CTT ACT GTT TTC AGA AAC ATT ATA ATT CAC

                                               330
TGT AAT GAT GTA ATT CTT GAA TAA TAA ATC TGA CAA AAT ATT (A)n
```

FIG. 4B

0217102

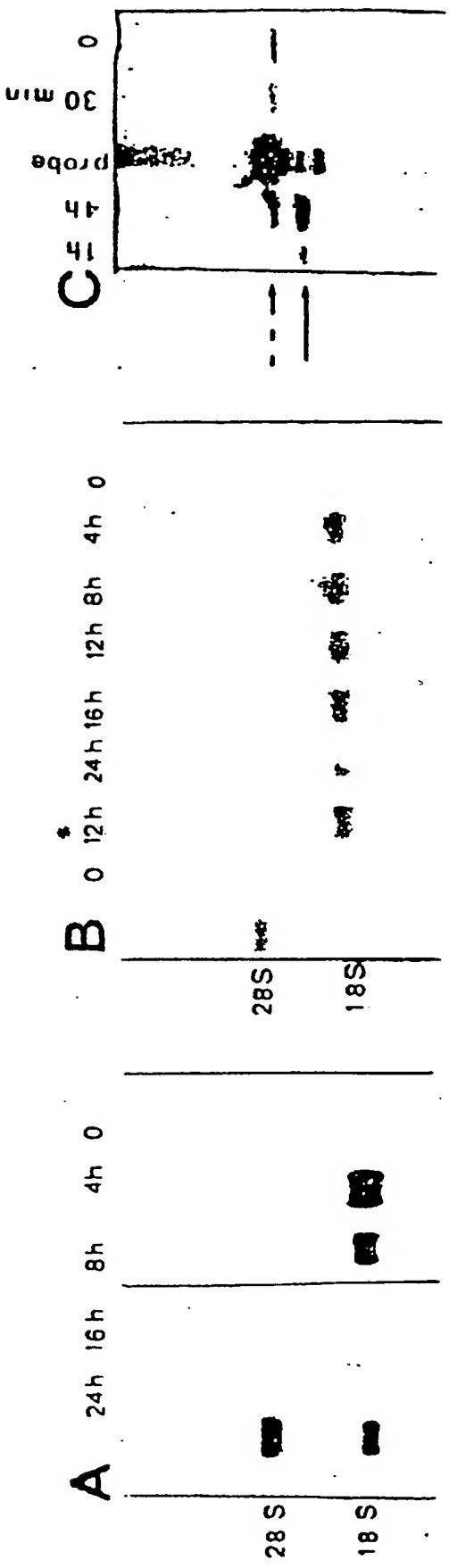

A
24h
16h
8h
4h
0
28 S
18 S
B
0
12h
24h
16h
8h
4h
0
28S
18S
C
41
41
probe
30 min
0

FIG.5

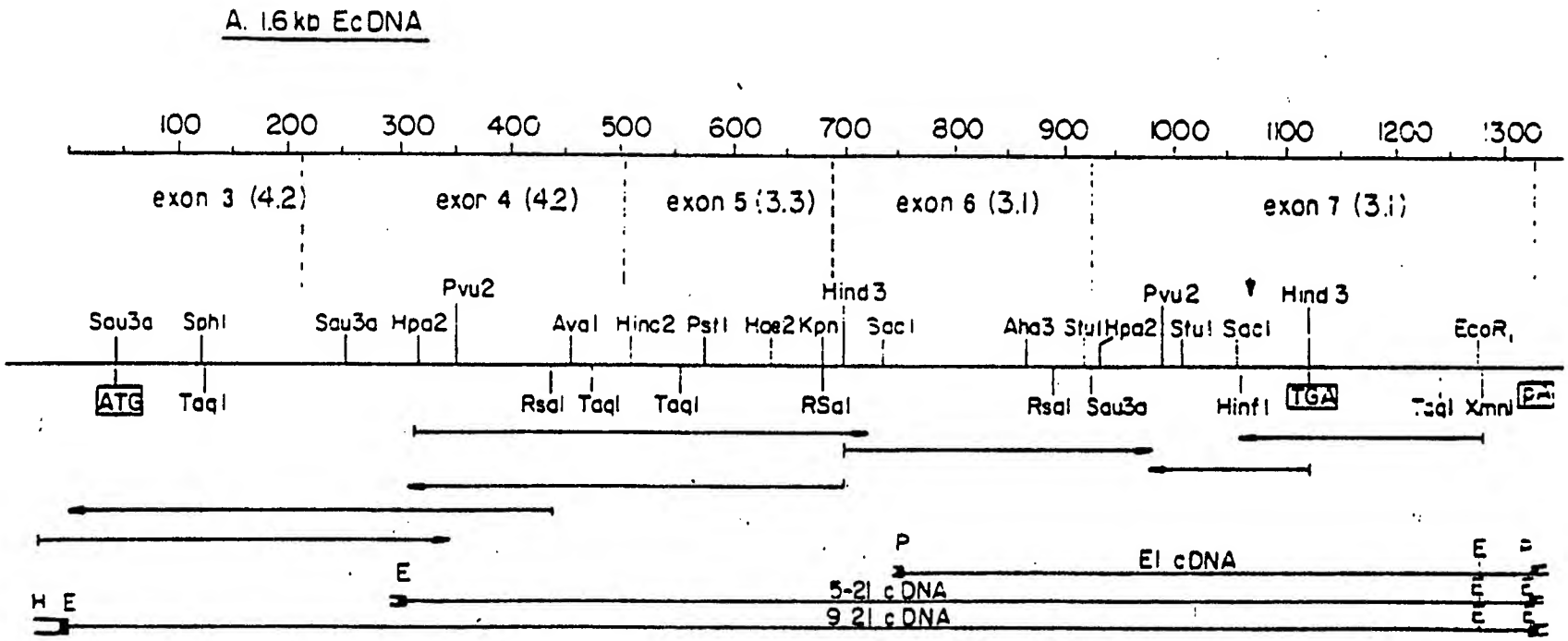


FIG.6A

0217102

B. 1.8kb EcDNA

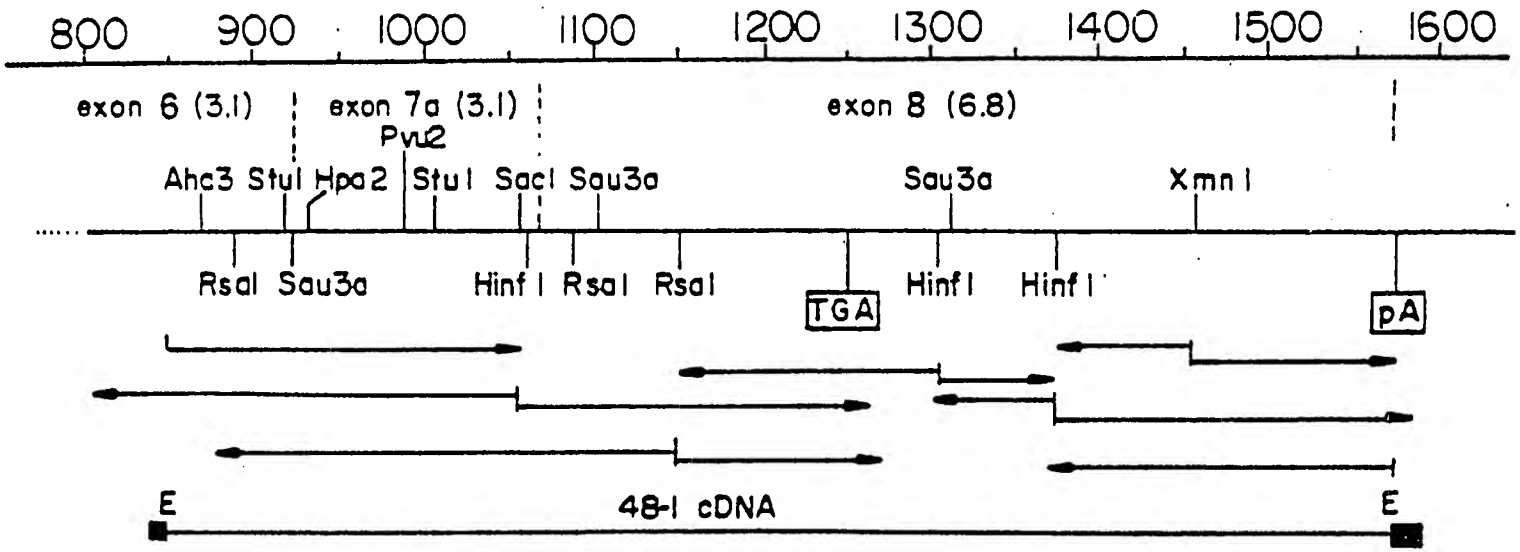


FIG. 6B

7A

SEQUENCE OF (2'-5') OLIGO A SYNTHETASE cDNA FCR 1.6 Kb RNA (E16,clcne 9-21)

FIG.7A

```
       1                                      30     (1)    Sau3a                60                                      90
E16..GAG GCA GTT CTG TTG CCA CTC TCT CTC CTG TCA ATG ATG GAT CTC AGA AAT ACC CCA GCC AAA TCT CTG GAC AAG TTC ATT GAA GAC TAT
                                                 MET MET ASP LEU ARG ASN THR PRO ALA LYS SER LEU ASP LYS PHE ILE GLU ASP TYR
     91                            Sph1      120     (31)                        150                                     180
E16  CTC TTG CCA GAC ACG TGT TTC CGC ATG CAA ATC GAC CAT GCC ATT GAC ATC ATC TGT GGG TTC CTG AAG GAA AGG TGC TTC CGA GGT AGC
     LEU LEU PRO ASP THR CYS PHE ARG MET GLN ILE ASP HIS ALA ILE ASP ILE ILE CYS GLY PHE LEU LYS GLU ARG CYS PHE ARG GLY SER
    181                    exon 3            210 |(61)       exon 4              240                    Sau3a                270
E16  TCC TAC CCT GTG TGT GTG TCC AAG GTG GTA AAG|GGT GGC TCC TCA GGC AAG GGC ACC ACC CTC AGA GGC CGA TCT GAC GCT GAC CTG GTT
     SER TYR PRO VAL CYS VAL SER LYS VAL VAL LYS GLY GLY SER SER GLY LYS GLY THR THR LEU ARG GLY ARG SER ASP ALA ASP LEU VAL
    271                                      300     (91)                        330                        Pvu2             360
E16  GTC TTC CTC AGT CCT CTC ACC ACT TTT CAG GAT CAG TTA AAT CGC CGG GGA GAG TTC ATC CAG GAA ATT AGG AGA CAG CTG GAA GCC TGT
     VAL PHE LEU SER PRO LEU THR THR PHE GLN ASP GLN LEU ASN ARG ARG GLY GLU PHE ILE GLN GLU ILE ARG ARG GLN LEU GLU ALA CYS
    361                                      390     (121)                       420                                     450
E16  CAA AGA GAG AGA GCA CTT TCC GTG AAG TTT GAG GTC CAG GCT CCA CGC TGG GGC AAC CCC CGT GCG CTC AGC TTC GTA CTG AGT TCG CTC
     GLN ARG GLU ARG ALA LEU SER VAL LYS PHE GLU VAL GLN ALA PRO ARG TRP GLY ASN PRO ARG ALA LEU SER PHE VAL LEU SER SER LEU
    451                                      480     (151)       exon 4      |exon 5 510                                     540
E16  CAG CTC GGG GAG GGG GTG GAG TTC GAT GTG CTG CCT GCC TTT GAT GCC CTG GGT CAG TTG ACT GGC AGC TAT AAA CCT AAC CCC CAA ATC
     GLN LEU GLY GLU GLY VAL GLU PHE ASP VAL LEU PRO ALA PHE ASP ALA LEU GLY GLN LEU THR GLY SER TYR LYS PRO ASN PRO GLN ILE
    541                                      570 Pst1(181)                       600                                     630
E16  TAT GTC AAG CTC ATC GAG GAG TGC ACC GAC CTG CAG AAA GAG GGC GAG TTC TCC ACC TGC TTC ACA GAA CTA CAG AGA GAC TTC CTG AAG
     TYR VAL LYS LEU ILE GLU GLU CYS THR ASP LEU GLN LYS GLU GLY GLU PHE SER THR CYS PHE THR GLU LEU GLN ARG ASP PHE LEU LYS
    631                                      660     (211)          Kpn1  exon 5 |690  exon 6 Hind3                          720
E16  CAG CGC CCC ACC AAG CTC AAG AGC CTC ATC CGC CTA GTC AAG CAC TGG TAC CAA AAT|TGT AAG AAG AAG CTT GGG AAG CTG CCA CCT CAG
     GLN ARG PRO THR LYS LEU LYS SER LEU ILE ARG LEU VAL LYS HIS TRP TYR GLN ASN CYS LYS LYS LYS LEU GLY LYS LEU PRO PRO GLN
    721             Sac1                     750     (241)                       780                                     810
E16  TAT GCC CTG GAG CTC CTG ACG GTC TAT GCT TGG GAG CGA GGG AGC ATG AAA ACA CAT TTC AAC ACA GCC CAA GGA TTT CGG ACG GTC TTG
     TYR ALA LEU GLU LEU LEU THR VAL TYR ALA TRP GLU ARG GLY SER MET LYS THR HIS PHE ASN THR ALA GLN GLY PHE ARG THR VAL LEU
    811                                      840     (271)              Aha3 870                                             900
E16  GAA TTA GTC ATA AAC TAC CAG CAA CTC TGC ATC TAC TGG ACA AAG TAT TAT GAC TTT AAA AAC CCC ATT ATT GAA AAG TAC CTG AGA AGG
     GLU LEU VAL ILE ASN TYR GLN GLN LEU CYS ILE TYR TRP THR LYS TYR TYR ASP PHE LYS ASN PRO ILE ILE GLU LYS TYR LEU ARG ARG
    901                exon 6 |exon 7  Sau3a 930     (301)                       960                                Pvu2 990
E16  CAG CTC ACG AAA CCC AGG CCT GTG ATC CTG GAC CCG GCG GAC CCT ACA GGA AAC TTG GGT GGT GGA GAC CCA AAG GGT TGG AGG CAG CTG
     GLN LEU THR LYS PRO ARG PRO VAL ILE LEU ASP PRO ALA ASP PRO THR GLY ASN LEU GLY GLY GLY ASP PRO LYS GLY TRP ARG GLN LEU
    991                                      1020    (331)                       1050  Sac1                               1080
E16  GCA CAA GAG GCT GAG GCC TGG CTG AAT TAC CCA TGC TTT AAG AAT TGG GAT GGG TCC CCA GTG AGC TCC TGG ATT CTG CTG GTG AGA CCT
     ALA GLN GLU ALA GLU ALA TRP LEU ASN TYR PRO CYS PHE LYS ASN TRP ASP GLY SER PRO VAL SER SER TRP ILE LEU LEU VAL ARG PRO
   1081                                      1110    (361)    Hind3              1140                                    1170
E16  CCT GCT TCC TCC CTG CCA TTC ATC CCT GCC CCT CTC CAT GAA GCT TGA GAC ATA TAG CTG GAG ACC ATT CTT TCC AAA GAA CTT ACC TCT
     PRO ALA SER SER LEU PRO PHE ILE PRO ALA PRO LEU HIS GLU ALA END
   1171                                      1200                                1230                                    1260
E16  TGC CAA AGG CCA TTT ATA TTC ATA TAG TGA CAG GCT GTG CTC CAT ATT TTA CAG TCA TTT TGG TCA CAA TCG ACG GTT TCT GGA ATT TTC
   1261                   EcoR1-Xmn1         1290                                1320                                    1350
E16  ACA TCC CTT GTC CAG AAT TCA TTC CCC TAA GAG TAA TAA TAA ATA ATC TCT AAC ACCAAAAA...
```

7B SEQUENCE OF cDNA FOR E 1.8 Kb RNA (E18, clone 46-1)

```
   901            exon 6 |exon 7a Sau3a 930    (301)                          960                                  Pvu2 990
E18..CAG CTC ACG AAA CCC AGG CCT GTG ATC CTG GAC CCG GCG GAC CCT ACA GGA AAC TTG GGT GGT GGA GAC CCA AAG GGT TGG AGG CAG CTG
  ..GLN LEU THR LYS PRC ARG PRC VAL ILE LEU ASP PRC ALA ASP PRC THR GLY ASN LEU GLY GLY GLY ASP PRO LYS GLY TRP ARG GLN LEU
   ---------------------------------------------------------------------------------------------------------------------
   991                                  1020    (331)                          1050   Sac1          exon 7a |exon 8 1080
E18 GCA CAA GAG GCT GAG GCC TGG CTG AAT TAC CCA TGC TTT AAG AAT TGG GAT GGG TCC CCA GTG AGC TCC TGG ATT CTG CTG|GCT GAA AGC
    ALA GLN GLU ALA GLU ALA TRP LEU ASN TYR PRC CYS PHE LYS ASN TRP ASP GLY SER PRC VAL SER SER TRP ILE LEU LEU ALA GLU SER
   ---------------------------------------------------------------------------------------------------------------------
  1081 (glycos.)                 Sau3a 1110    (361)                          1140                                  1170
E18 AAC AGT ACA GAC GAT GAG ACC GAC GAT CCC AGG ACG TAT CAG AAA TAT GGT TAC ATT GGA ACA CAT GAG TAC CCT CAT TTC TCT CAT AGA
    ASN SER THR ASP ASP GLU THR ASP ASP PRC ARG THR TYR GLN LYS TYR GLY TYR ILE GLY THR HIS GLU TYR PRC HIS PHE SER HIS ARG
  1171                                 1200    (391)                          1230                                  1260
E18 CCC AGC ACG CTC CAG GCA GCA TCC ACC CCA CAG GCA GAA GAG GAC TGG ACC TGC ACC ATC CTC TGA ATG CCA GTG CAT CTT GGG GGA AAG
    PRC SER THR LEU GLN ALA ALA SER THR PRC GLN ALA GLU GLU ASP TRP THR CYS THR ILE LEU END
  1261                                 1290                          Sau3a 1320                                  1350
E18 GGC TCC AGT GTT ATC TGG ACC AGT TCC TTC ATT TTC AGG TGG GAC TCT TGA TCC AGA GAA GAC AAA GCT CCT CAG TGA GCT GGT GTA TAA
  1351                                 1380                                 1410                                  1440
E18 TCC AAG ACA GAA CCC AAG TCT CCT GAC TCC TGG CCT TCT ATG CCC TCT ATC CTA TCA TAG ATA ACA TTC TCC ACA GCC TCA CTT CAT TCC
  1441              Xmn1               1470                                 1500                                  1530
E18 ACC TAT TCT CTG AAA ATA TTC CCT GAG AGA GAA CAG AGA GAT TTA GAT AAG AGA ATG AAA TTC CAG CCT TGA CTT TCT TCT GTG CAC CTG
  1531                                 1560                                 1590                                  1620
E18 ATG GGA GGG TAA TGT CTA ATG TAT TAT CAA TAA CAA TAA AAA TAA AGC AAA TAC CAAAAA...
```

FIG. 7B

0217102

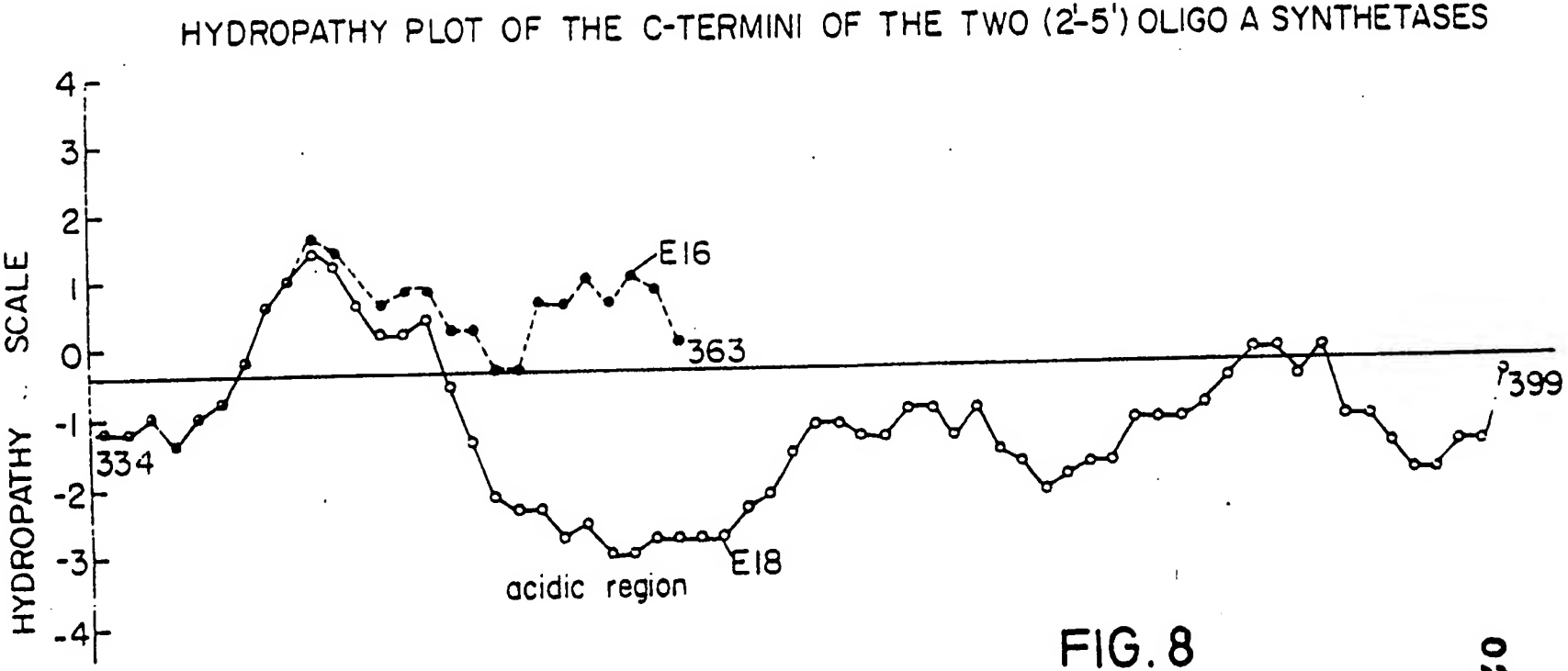


FIG. 8

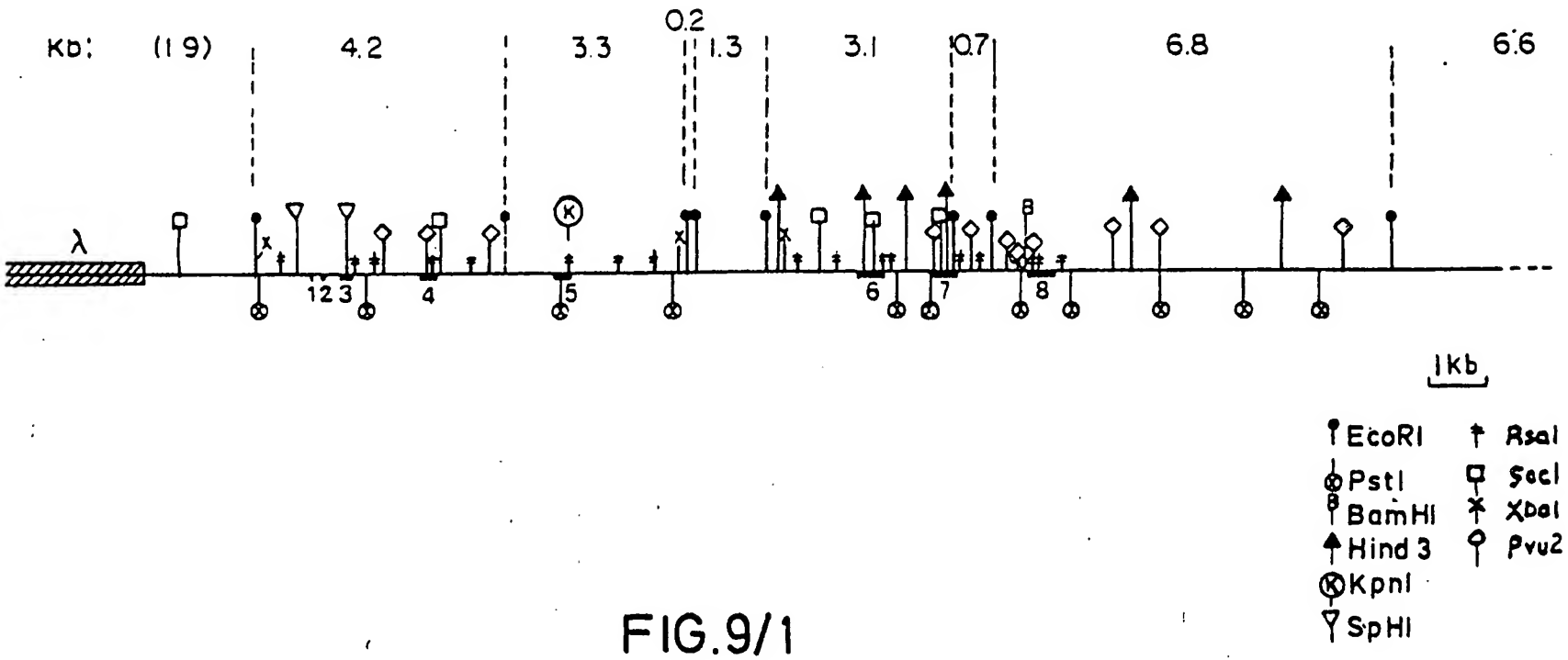


FIG.9/1

0217102

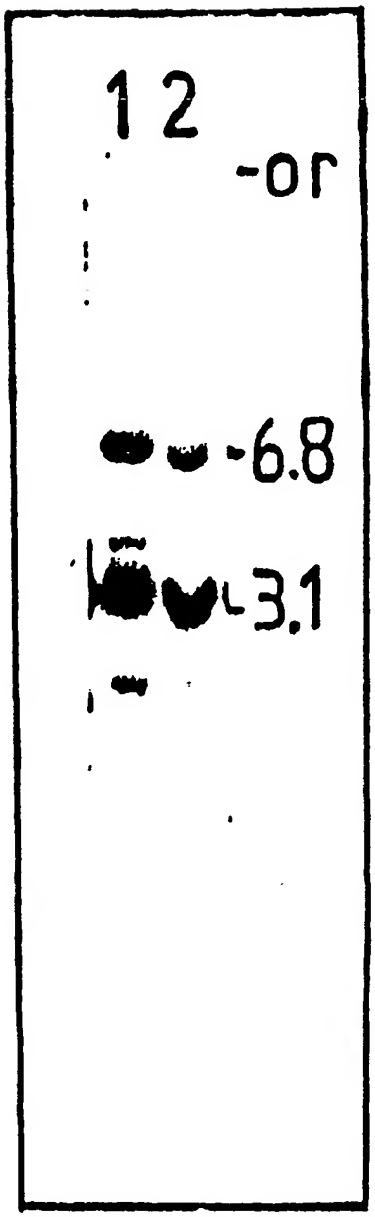


FIG.9/2

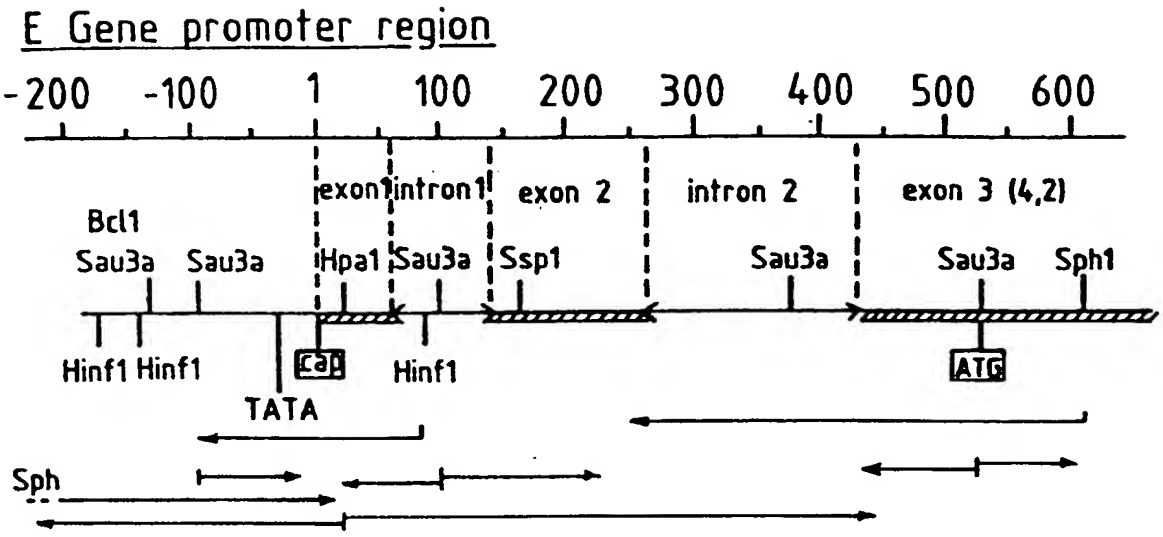

E Gene promoter region
-200
-100
1
100
200
300
400
500
600
exon1
intron1
exon 2
intron 2
exon 3 (4,2)
Bcl1
Sau3a
Sau3a
Hpa1
Sau3a
Ssp1
Sau3a
Sau3a
Sph1
Hinf1
Hinf1
CAP
Hinf1
ATG
TATA
Sph

FIG.10

PROMOTER REGION OF THE HUMAN (2'-5') OLIGO A SYNTHETASE GENE

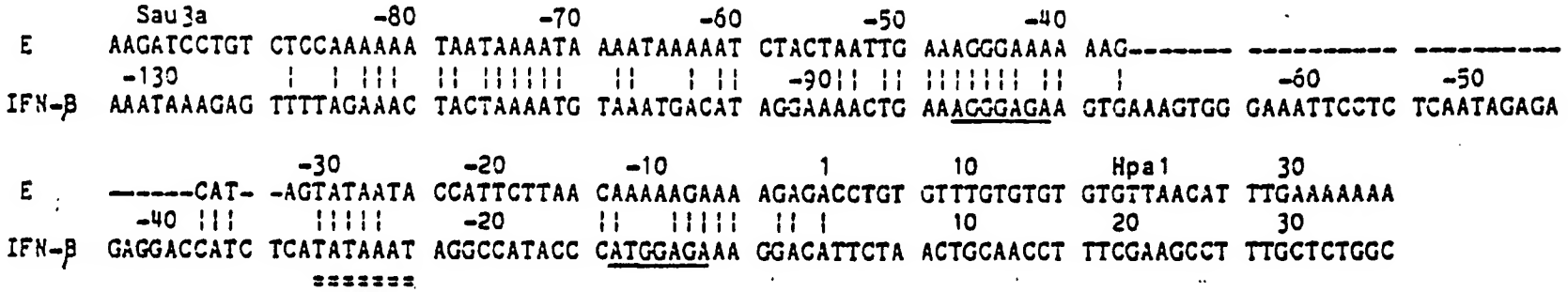
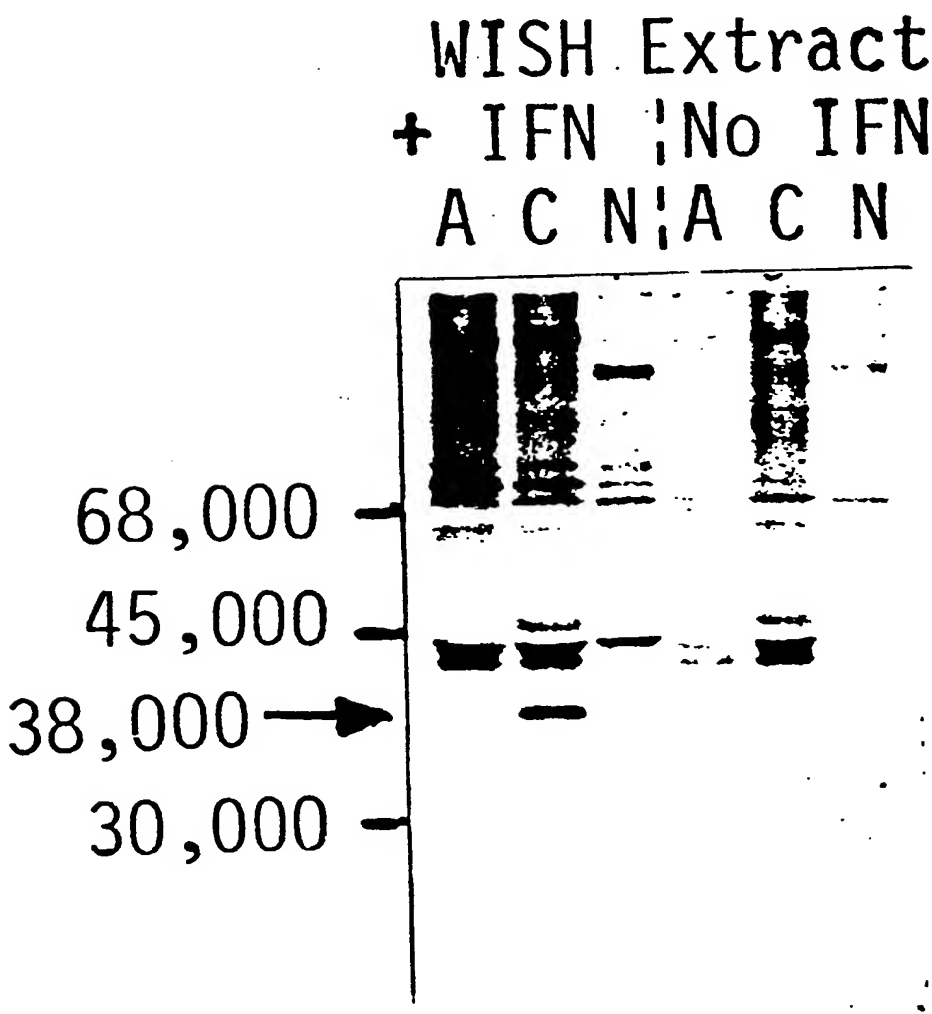


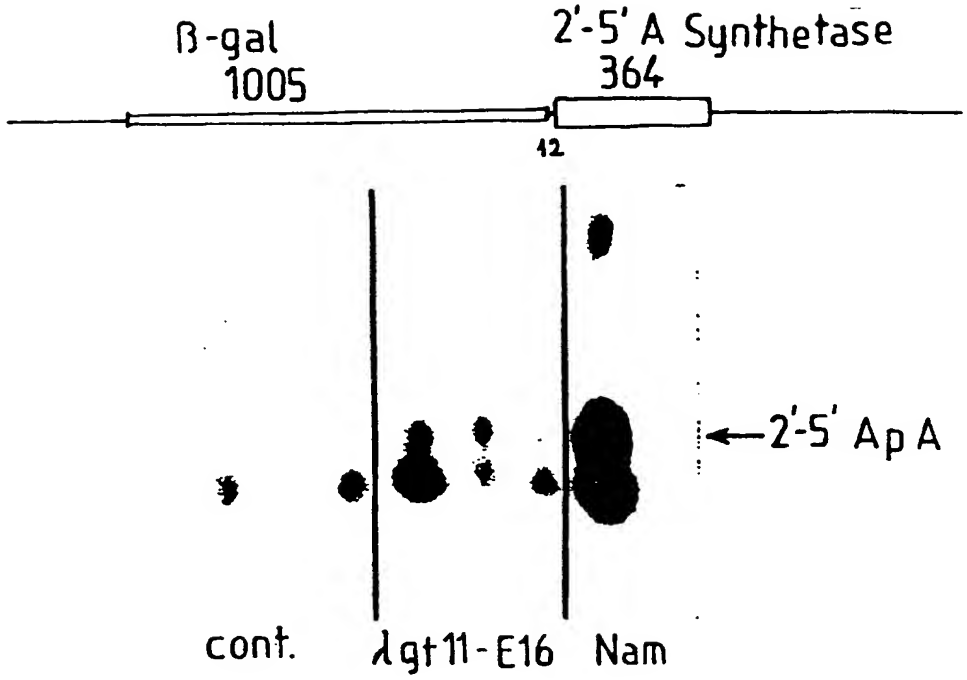
FIG. 11

Immunoprecipitation of (2'-5')oligoA Synthetase



A: Peptide 284-303
B: Peptide 348-364 E16
N: Non-immune serum

FIG. 12



E cDNA EXPRESSION IN E. coli

FIG.13

QUICK CELL BLOT FOR HEASURE OF (2'-5') OLIGO A SYNTHETASE RNA

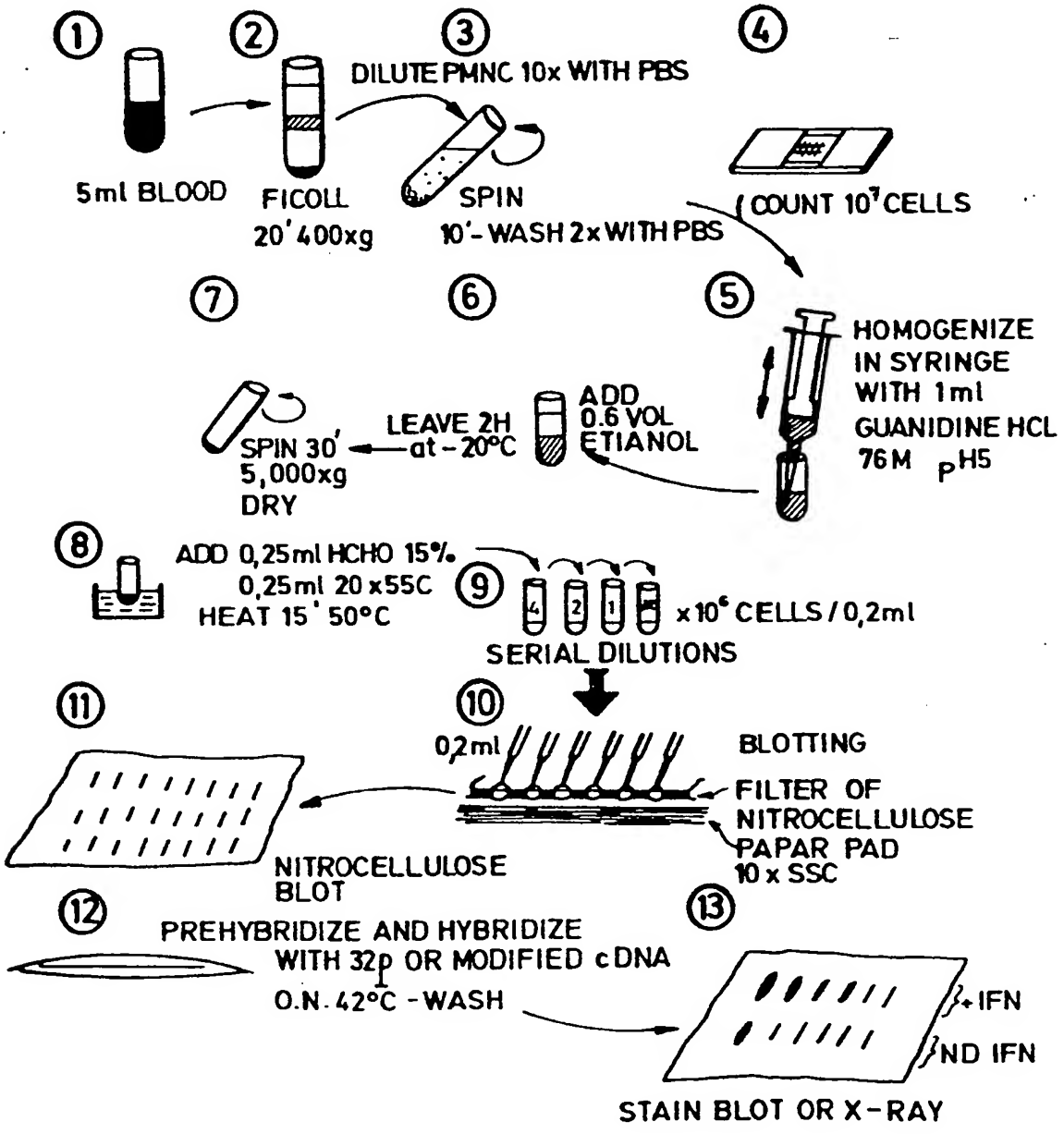


FIG. 14

QUICK CELL BLOT FOR (2'-5') A SYNTHETASE RNA

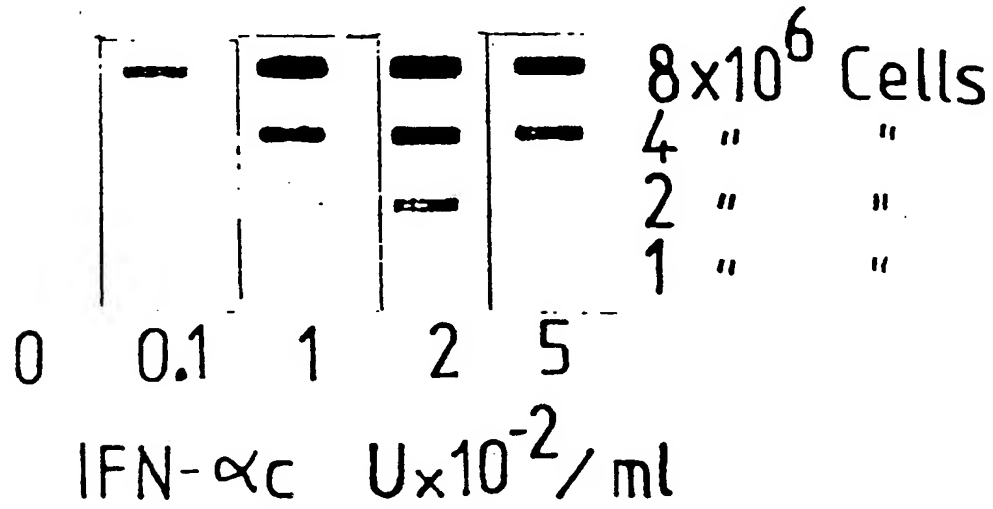


FIG. 15

SPECIFIC IMMUNOPRECIPITATION OF (2'-5') A SYNTHETASE ACTIVITY BY ANTI-PEPTIDE ANTIBODIES

ENZYME ACTIVITY IMMUNOPRECIPITATED FROM			
DAUDI CELLS		WISH CELLS	
ANTI-B cpm	ANTI-C cpm	ANTI-B cpm	ANTI-C cpm
5,600	0	12,420	48,290

mean of three experiments

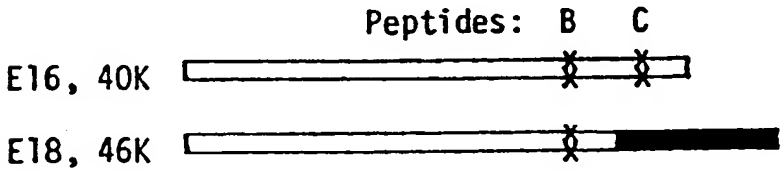
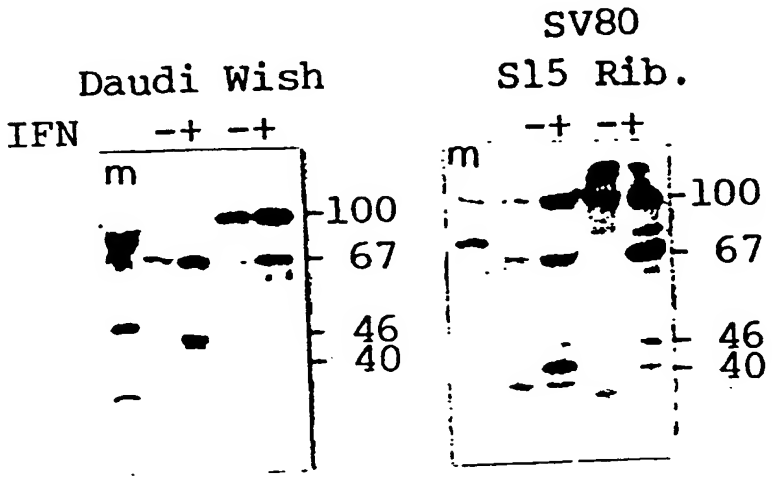


FIG.16

Different forms of (2'-5') A synthetase
in human cells and subfractions



Western blot
Anti OASE-B

FIG. 17

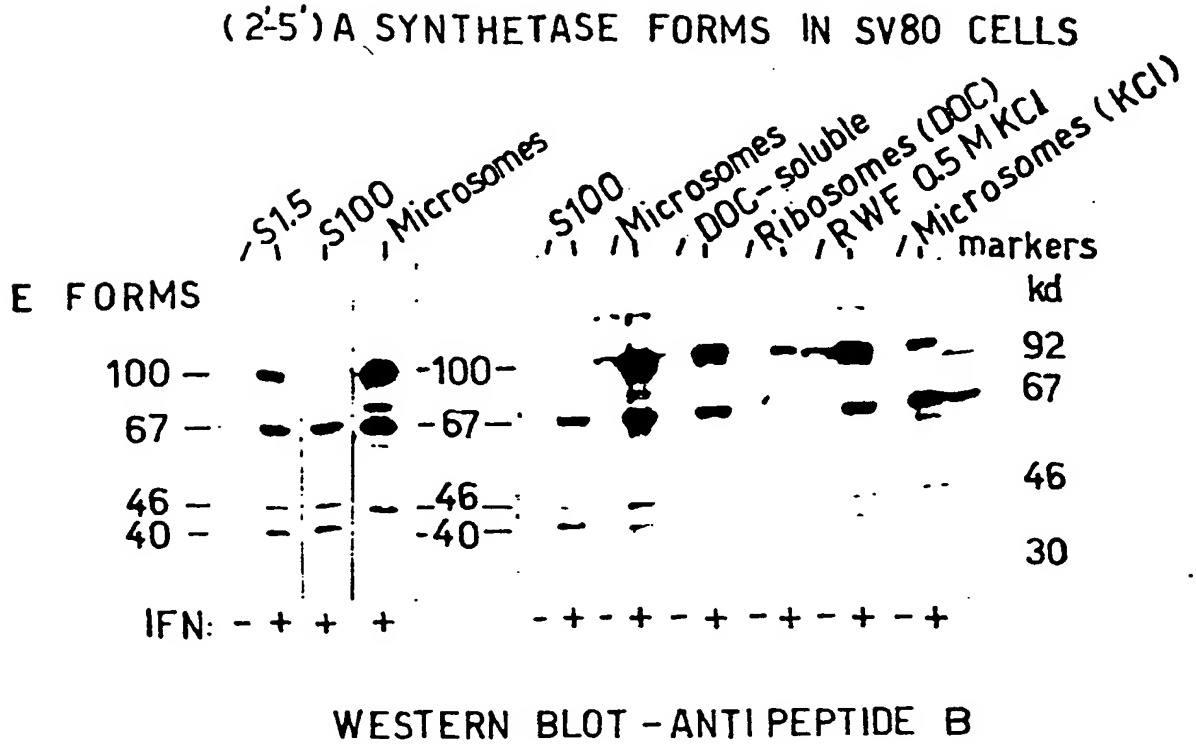
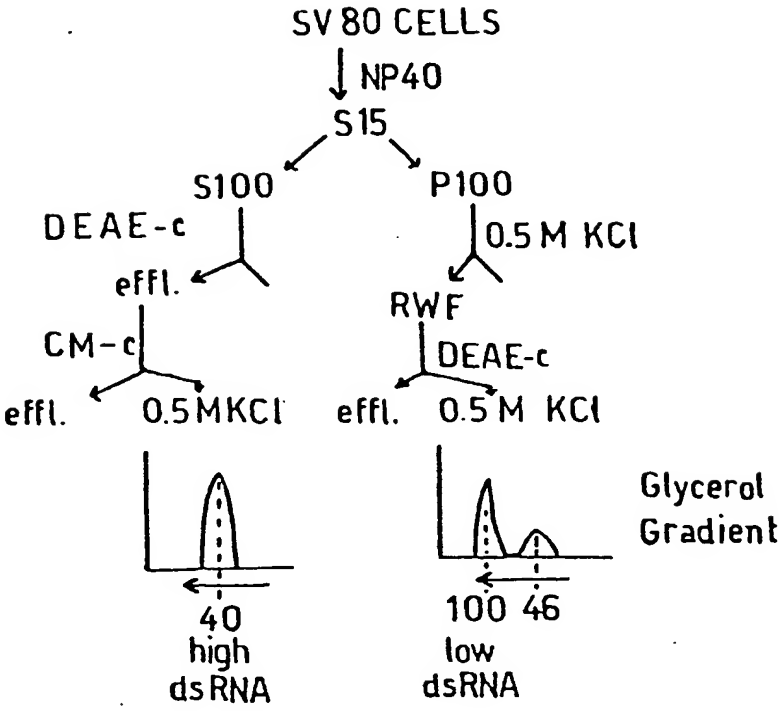
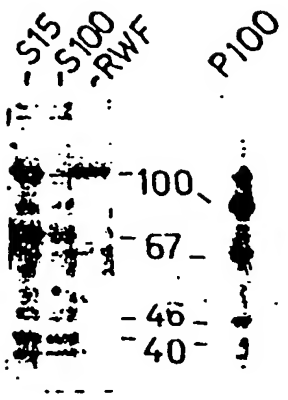
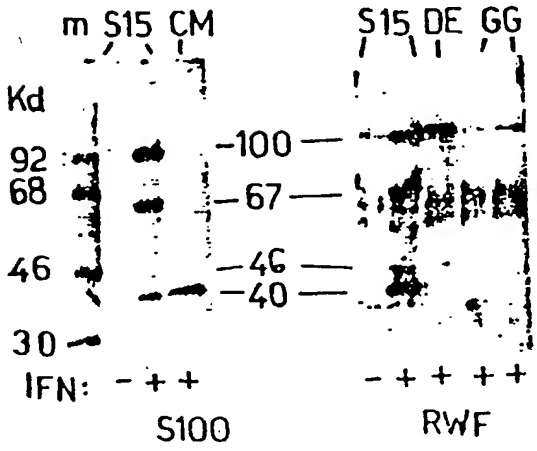

(2'-5') A SYNTHETASE FORMS IN SV80 CELLS
S1.5
S100
Microsomes
S100
Microsomes
DOC-soluble
Ribosomes (DOC)
RWF 0.5 M KCl
Microsomes (KCl)
markers
kd
E FORMS
100
67
46
40
-100-
-67-
-46-
-40-
92
67
46
30
IFN: - + + + - + - + - + - + - + - +
WESTERN BLOT - ANTI PEPTIDE B

FIG. 18

<4127







INTRACELLULAR LOCALISATION OF 4 FORMS OF 2-5' A SYNTHETASE

(Western blots) anti-B

FIG.19

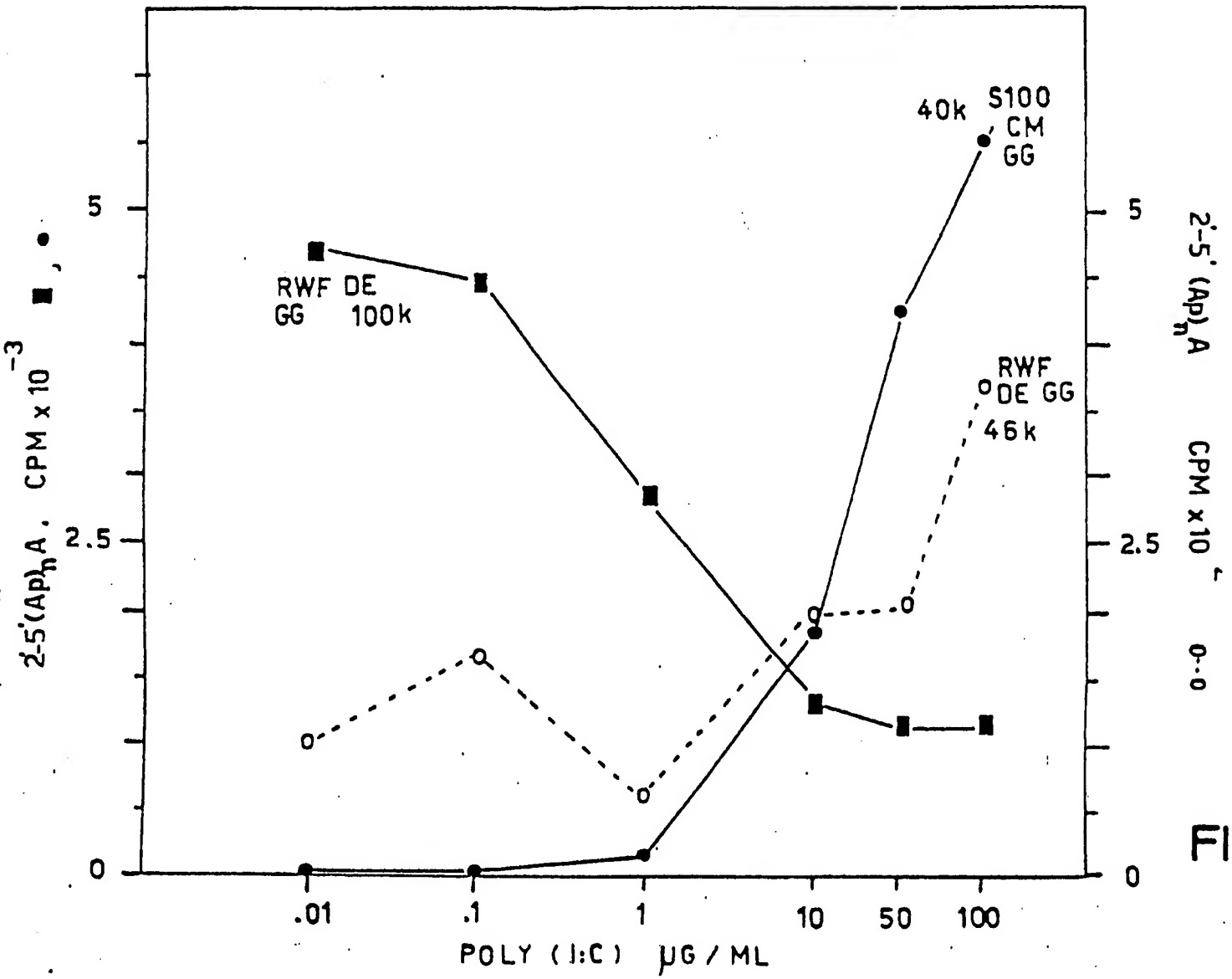

40k S100
CM
GG
RWF DE
GG 100k
RWF
DE GG
46k
5
2.5
0
2'-5' (Ap)nA CPM x 10-3 ■, ●
2'-5' (Ap)nA CPM x 10-3 o-o
.01
.1
1
10
50
100
POLY (I:C) µG / ML

FIG. 20

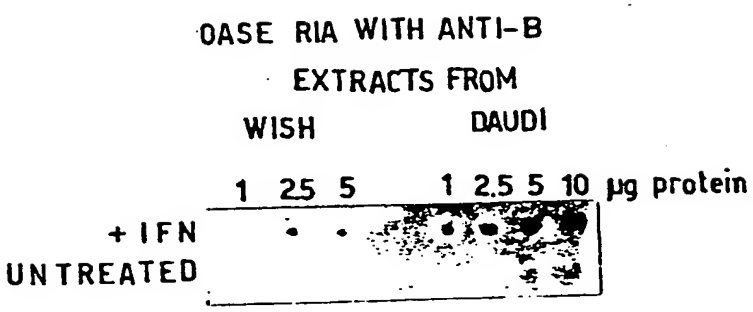

OASE RIA WITH ANTI-B
EXTRACTS FROM
WISH
DAUDI
1 25 5
1 2.5 5 10 µg protein
+ IFN
UNTREATED

FIG. 21

DIAGNOSIS OF VIRAL INFECTION BY IMMUNOFLUORESCENCE STAINING OF PERIPHERAL BLOOD MONONUCLEAR CELLS WITH ANTI-OASE SERUM

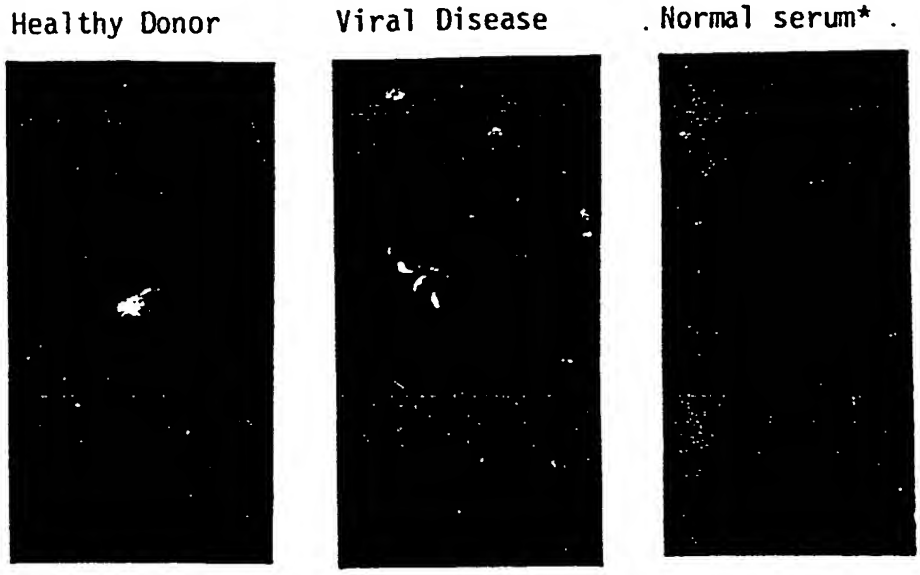


FIG.22

European Patent Office

EUROPEAN SEARCH REPORT

Application number

EP 86111585.5

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl 4)
P,X	THE EMBO JOURNAL, vol. 4, no. 9, 1985 (Oxford, GB) P. BENECH et al. "Structure of two forms of the interferon-induced (2'-5') oligo A synthetase of human cells based on cDNAs and gene sequences" pages 2249-2256 * Totality * --	1-15, 25	C 12 N 15/00 C 07 H 21/04 C 07 H 21/02 C 12 N 1/20 C 12 N 9/00 C 12 Q 1/00 G 01 N 33/53 C 07 K 7/10 A 61 K 39/395 //(C 12 N 1/20; C 12 R 1:19)
D,X	NUCLEIC ACIDS RESEARCH, vol. 13, no. 4, February 25, 1985 (Oxford, GB) P. BENECH et al. "3'End structure of the human (2'-5') oligo A synthetase gene; prediction of two distinct proteins with cell type-specific expression" pages 1267-1281 * Totality * --	1,6,7, 25	
D,X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, vol. 80, no. 16, August 1983 (Baltimore, USA) G. MERLIN et al. "Molecular cloning and sequence of partial cDNA for interferon-induced (2'-5') oligo (A) synthetase mRNA from human cells" pages 4904-4908 * Totality * ----	1,25	

TECHNICAL FIELDS SEARCHED (Int Cl 4)

C 12 N
C 07 H
C 12 Q
G 01 N
C 07 K
A 61 K

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
VIENNA	01-12-1986	WOLF

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO Form 1503 03 82

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CC   predicting disease complications in an individual or monitoring response
CC   to treatment in an individual. The diseases include cardiac allograft
CC   rejection, kidney allograft rejection, liver allograft rejection,
CC   atherosclerosis, congestive heart failure, systemic lupus erythematosus,
CC   rheumatoid arthritis, osteoarthritis or cytomegalovirus infection
XX
SQ   Sequence 50 BP; 11 A; 11 C; 15 G; 13 T; 0 U; 0 Other;

  Query Match             100.0%;  Score 50;  DB 6;  Length 50;
  Best Local Similarity   100.0%;  Pred. No. 1.1e-10;
  Matches   50;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 CTGAGACTGGCTGCTGACTTTGAGAACTCTGTGAGACAAGGTCCTTAGGC 50
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 CTGAGACTGGCTGCTGACTTTGAGAACTCTGTGAGACAAGGTCCTTAGGC 50


RESULT 2
AAN70147
ID   AAN70147 standard; cDNA; 342 BP.
XX
AC   AAN70147;
XX
DT   18-APR-1991  (first entry)
XX
DE   Sequence of cDNA clone C56 corresponding to an interferon-induced mRNA.
XX
KW   In vivo interferon assay; ss.
XX
OS   Homo sapiens.
XX
FH   Key             Location/Qualifiers
FT   CDS             1. .201
FT                   /*tag=  a
FT   polyA_signal    322. .328
FT                   /*tag=  c
FT   polyA_site      343
FT                   /*tag=  b
XX
PN   EP217102-A.
XX
PD   08-APR-1987.
XX
PF   21-AUG-1986;    86EP-00111585.
XX
PR   28-AUG-1985;    85IL-00076233.
PR   08-APR-1986;    86IL-00078445.
XX
PA   (YEDA ) YEDA RES & DEV CO LTD.
XX
PI   Revel M,  Chebath J;
XX
DR   WPI; 1987-095196/14.
DR   P-PSDB; AAP70093.
XX
PT   Recombinant enzyme having (2'-5') oligo A synthetase activity - used for
PT   monitoring the response of a patient to an interferon.
XX
PS   Example; Fig 4B; 90pp; English.
XX
CC   A partial cDNA clone (E1) for the OAS mRNA from human SV80 cells was
CC   first obtd. through its ability to select by hybridisation a mRNA
CC   producing OAS activity upon translation in Xenopus laevis oocytes. The E1
CC   cDNA insert hybridises to 3RNA species of 1.6, 1.8 and 3.6 kb which are
CC   coinduced by IFN in SV80 cells. cDNA clones for the 1.6 and 1.8 RNAs have
CC   been isolated and sequenced
XX
SQ   Sequence 342 BP; 108 A; 59 C; 66 G; 109 T; 0 U; 0 Other;

  Query Match             100.0%;  Score 50;  DB 1;  Length 342;
  Best Local Similarity   100.0%;  Pred. No. 2e-10;
  Matches   50;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 CTGAGACTGGCTGCTGACTTTGAGAACTCTGTGAGACAAGGTCCTTAGGC 50
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db        154 CTGAGACTGGCTGCTGACTTTGAGAACTCTGTGAGACAAGGTCCTTAGGC 203


RESULT 3
AAT21101
ID   AAT21101 standard; cDNA to mRNA; 408 BP.
XX
AC   AAT21101;
XX
DT   16-JUL-1996  (first entry)
XX
DE   Human gene signature HUMGS02403.
XX
KW   Gene signature; messenger RNA; mRNA; relative abundance; frequency;
KW   human; cloning; mapping; non-biased library; diagnosis; detection;
KW   cell typing; abnormal cell function; ss.
XX
OS   Homo sapiens.
XX
PN   WO9514772-A1.
XX
PD   01-JUN-1995.
XX
PF   11-NOV-1994;   94WO-JP001916.
XX
PR   12-NOV-1993;   93JP-00355504.
XX
PA   (MATS/) MATSUBARA K.
PA   (OKUB/) OKUBO K.
XX
PI   Matsubara K,  Okubo K;
XX
DR   WPI; 1995-206931/27.
XX
PT   Single-stranded DNA for identifying gene signatures - isolated from 3'-
PT   directed human cDNA library that reflects relative abundance of corresp.
PT   mRNA in specific human tissues.
XX
PS   Claim 1; Page 800; 2245pp; Japanese.
XX
CC   A single-stranded DNA (or its complementary strand or the corresp. double
CC   -stranded DNA) which comprises one of the 7837 "GS" sequences given in
CC   AAT19001-T26837 and which is able to hybridise to part of human genomic
CC   DNA, cDNA or mRNA is claimed. The GS (Gene Signature) sequences were
CC   obtained from 3'-directed cDNA libraries prepared from various human
CC   tissues; synthesis of cDNA was initiated from the 3'-end of mRNA by using
CC   poly(T) as the sole primer. Since the 3'- untranslated sequence is unique
CC   to a particular mRNA species, almost all the 3'-oriented cDNAs hybridise
CC   with specific mRNAs. Each library is constructed so as to reflect
CC   accurately the relative abundance of different mRNAs in the particular
CC   tissue from which it was derived. The appearance frequency of a given GS
CC   in a cDNA library can be determined (esp. using primers and probes
CC   derived from the GS sequences) as a means of diagnosing abnormal cell
CC   function or for recognising different cell types
XX
SQ   Sequence 408 BP; 133 A; 64 C; 80 G; 130 T; 0 U; 1 Other;

  Query Match             100.0%;  Score 50;  DB 2;  Length 408;
  Best Local Similarity   100.0%;  Pred. No. 2.1e-10;
  Matches   50;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 CTGAGACTGGCTGCTGACTTTGAGAACTCTGTGAGACAAGGTCCTTAGGC 50
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         85 CTGAGACTGGCTGCTGACTTTGAGAACTCTGTGAGACAAGGTCCTTAGGC 134


RESULT 4
ABV15983
ID   ABV15983 standard; cDNA; 418 BP.
XX
AC   ABV15983;
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